The effects of 5 days β-alanine supplementation on the velocity and the percentage $\dot{V}o_{2max}$ at the lactate threshold.

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Declaration

The work is original and has not been previously submitted in support of a degree qualification or other course.

Signed……..

Date ……..

The effects of 5 days β-alanine supplementation on the velocity and the percentage □o_{2max} at the lactate threshold.

Abstract **Matthew Johnson**

Purpose: To assess the effect of 5 days β-alanine supplementation on the velocity and the percentage of ·O_{2max} (%·O_{2max}) at the lactate threshold (T_{lac}) during treadmill running.

Method: Using a double-blind, placebo-control, repeated measures, cross-over design, 6 participants (4 male, 2 female) undertook a T_{lac} test using a motorised treadmill on 3 separate occasions. For 5 days prior to each test participants ingested β-alanine (50mg·kg-1·d-1) or an equal amount of maltodextrin (placebo). Participants performed a control trial where no supplements were ingested prior to testing (control). The velocity, %·O_{2max}, ·O_2, blood lactate, heart rate (HR) and RPE at the T_{lac} were measured during each trial. Significant differences between the 3 trials were assessed via one-way repeated-measures analysis of variance ($p \leq 0.05$).

Results: There was no significant difference ($p = 0.369$) in the velocity at the T_{lac} between the β-alanine (10.17 ± 0.98 km·h^{-1}), placebo (10.33 ± 1.03 km·h^{-1}) or the control trials (10.83 ± 1.16km·h^{-1}). There was no significant difference ($p = 0.087$) in %·O_{2max} at the T_{lac} between the β-alanine (75.21 ± 6.84%), placebo (75.93 ± 7.32%) or control trials (69.94 ± 7.39). This was coupled with a non significant difference in ·O_2 between the 3 trials ($p = 0.103$). Blood lactate was not significantly different ($p = 0.628$) between the β-alanine, placebo and control trials (4.1 ± 1.2, 3.7 ± 1.5 and 4.0 ± 1.8mmol·L, respectively). HR and RPE at the T_{lac} were unchanged between trials ($p > 0.05$).

Conclusion: The data suggests that 5 days β-alanine supplementation has no effect on the velocity or %·O_{2max} at the T_{lac} during treadmill running. A five day supplementation period may not be sufficient to augment muscle carnosine concentrations to elicit an ergogenic effect.

Keywords: NUTRITIONAL SUPPLEMENTS, β-ALANINE, LACTATE THRESHOLD, ENDURANCE PERFORMANCE.

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1. Introduction

For many years athletes of all levels have sought to gain an advantage over their competitors. Genetic endowment and an effective periodised training programme are without doubt imperative to success (Ahmetov, Williams, Popov et al., 2009; Ostrander, Huson & Ostrander, 2009). In attempts to attain success at the top level athletes have adopted all means necessary to boost their performance. Strategies like alternative training methods (Foster, Hector, Welsh et al., 1995), hypnotherapy (Caird, McKenzie & Sleivert, 1999), special diets (Burke, Keins & Ivy, 2001), the use of new equipment (Nigg, Stefanyshyn, Cole, Stergiou & Miller, 2003) or nutritional supplements (Maughan, King & Lea, 2004), are but a few examples. At elite level genetic ability and training techniques are likely to be maximised and, therefore, any means of gaining an advantage need to be explored. One avenue of exploration open to athletes is the use of nutritional supplements. A substance taken with the intent of improving performance or enhancing physical working capacity is referred to as being ergogenic (McArdle, Katch & Katch, 2007; Maughan, 1999). There are hundreds of proposed ergogenic supplements. Few, however, are supported by clinical trials demonstrating the ergogenic effect of these substances. The use of supplementation in athletes is widespread (Braun, Koehler, Geyer, Kleiner, Mester, et al., 2009). Many athletes chose to use supplements based on marketing claims and myths, without being aware of whether the substance has been scientifically proven to be ergogenic (Maughan, Depiesse & Geyer, 2007). Such actions may actually impede performance and more importantly result in serious health consequences. Therefore, it is essential that new supplements are clinically trialled by scientists, academics and researchers before being recommended to athletes and coaches. In addition to protecting the health of athletes, testing new substances could expand current knowledge in

physiology and biochemistry and help determine how these substances exert their ergogenic effect.

Nutritional ergogenic supplements are taken with one goal in mind – to improve performance. How they achieve this ultimate goal may differ depending on the substance in question. For example, some supplements are taken to enhance physiological adaptation following training; while others are taken to enhance energy availability or utilisation. The enhancement of energy availability would inevitably delay fatigue and augment physical working capacity. Alternatively, fatigue can be delayed by supplementing with a substance that reduces the concentrations of metabolic by-products from energy delivery. These metabolic by-products alter the internal environment which results in attenuations of muscle performance (Allen, Lamb & Westerblad, 2008).

2. Literature Review

2.1 Fatigue

Maintaining cellular homeostasis, specifically acid-base balance, becomes increasingly problematic during high-intensity exercise due to the amplified rate of lactate and/or proton release as the anaerobic glycolytic system begins to predominate (Spriet, Howlett & Heigenhauser, 2000). In an order to attempt to maintain acid-base balance several chemical buffering systems exist. These are divided into extracellular and intracellular buffers. Extracellular buffers include bicarbonate, plasma phosphates and plasma proteins. Intracellular buffers include histidine containing dipeptides (HCD), organic and inorganic phosphates and haemoglobin in the erythrocyte (Burton & Post, 2001). Scientists traditionally understood that lactate production was a consequence of a reduced oxygen supply forcing the muscle into anaerobic

metabolism (Katz & Sahlin, 1988). However, research over the last three to four decades has questioned traditional belief, and suggests that aerobic metabolism produces lactate when exercising at intensities of above 50 percent maximum oxygen uptake ($\dot{V}O_{2max}$) (Gladden, 2004; Spriet et al., 2000). During light exercise (< 50 percent $\dot{V}O_{2max}$) pyruvate and the reduced equivalent of nicotinamide adenine dinucleotide (NAD^+), NADH, are transported into the mitochondria for complete oxidation, and therefore lactate production is low. As exercise intensity increases so does the demand for adenosine triphosphate (ATP), thereby increasing the reliance on glycolytic ATP production. The production of NADH and pyruvate are increased and the capacity of the transport systems for pyruvate and NADH become exceeded. In a bid to maintain the glycolytic rate NAD^+ needs to be continually regenerated in order to accept the H^+ formed from the reduction of phosphoglyceraldehyde. As the concentration of NAD^+ becomes limited, the reduction of pyruvate to lactic acid is augmented as it provides a means of preventing the build up of H^+ in the cytosol. Consequently, lactate concentrations begin to rise. In solution lactic acid disassociates forming the salt sodium lactate and H^+ (Robergs, 2000). The rise in H^+ causes decreases in intramuscular pH which interfere with the excitation-contraction coupling process of skeletal muscle, which, in turn, leads to decreases in power output and fatigue (Fitts & Holloszy, 1976). The implication of lactate in fatigue is still the topic of debate, with recent work suggesting that lactate has no direct impact on fatigue (Robergs, Ghiasvand & Parker, 2004). The concomitant increase in lactate and H^+ does not imply cause and effect. Whether or not lactate is the cause of fatigue *per se*, lactate production during exercise provides an indication of glycolytic ATP production and a marker of H^+ accumulation. It can therefore be used as a relative marker of exercise intensity and fatigue.

2.2 Endurance performance

Endurance running performance is dependent on a high rate of oxidative phosphorylation for the resynthesis of ATP. Success during endurance running requires the integration of cardiovascular, neurological and metabolic factors, and the cooperation of these systems to transfer ATP into the highest attained velocity (Coyle, 2007; Hawley & Spargo, 2007; Spriet, 2007). Several physiological variables have been identified as strong determinants of endurance running performance. The resultant integration of these physiological variables is to attain the highest velocity (the performance velocity), whilst maintaining a high rate of oxidative ATP production (Coyle, 1995, Coyle, Joyner & Coyle, 2008). Three important determinants of endurance running performance include $\dot{V}O_{2max}$, the lactate threshold (T_{lac}) and running economy. During long distance races energy is predominantly derived from oxidative ATP production. $\dot{V}O_{2max}$ therefore has obvious importance as this represents the upper limit for aerobic energy production (Basset & Howley, 2000). Several investigations have identified a relationship between $\dot{V}O_{2max}$ and endurance running performance (Foster, 1983; Hagan, Smith & Gettman, 1981). Nevertheless, other physiological variables have been suggested to be stronger determinants of endurance performance (Costill, Branam, Eddy & Sparks, 1971; Davies & Thompson, 1979; Farrell, Wilmore, Coyle, Billing & Costill, 1979; Fay, Londeree, LaFontaine & Volek, 1989; Hagan, Upton, Duncan & Gettman, 1987; LaFontaine, Londeree & Spath, 1981). Another determinant of endurance running, and one that influences the performance velocity, is the velocity at T_{lac} (Coyle, 1999). The volume of oxygen ($\dot{V}O_2$) consumed at the T_{lac} has a direct impact on the velocity at T_{lac}. When the $\dot{V}O_2$ is expressed as a percentage of $\dot{V}O_{2max}$ it often coincides with the T_{lac} (Joyner & Coyle, 2008). The percentage of $\dot{V}O_{2max}$ at which the T_{lac} occurs gives

an indication of the pace that can be maintained for a prolonged period (Bassett & Howley, 2000)

2.3 Lactate threshold (T_{lac})

The T_{lac} can be defined as the exercise intensity (velocity or $\dot{V}O_2$) that precedes the non-linear accumulation in blood lactate concentrations (Ivy, Withers, Van Handel, Elger & Costill, 1980). The T_{lac} is measured during a graded exercise test (GXT) and a blood sample is taken at several different exercise intensities to assess lactate concentrations. These are then plotted on a graph against exercise intensity and used to determine the T_{lac}. The point at which lactate levels increase in a non-linear fashion from resting value represents the T_{lac} (Ivy et al., 1980). Over the years investigators have used a variety of methods to describe and identity the T_{lac}. For instance, Farrell et al. (1979) used the term "onset of plasma lactate accumulation" and identified this as the point on the graph directly preceding the exponential rise in plasma lactate. In contrast, Sjödin and Jacobs (1981) used a fixed lactate concentration of 4mmol·l^{-1} to identify this benchmark, while other laboratories (Allen, Seals, Hurley, Eshani & Hagberg, 1995) utilised a lactate concentration of 2.5 mmol·L^{-1}. While having a fixed blood lactate concentration makes identification of the T_{lac} easier, it limits individuality and further more, variations in lactate tolerance may mean a physiological threshold does not occur at exactly 4mmol·l^{-1} in all individuals (Bosquet, Léger & Legros, 2002). Another methodological approach utilised to pinpoint the T_{lac} is known as the D_{max} method (Cheng, Kuipers, Snyder et al., 1992). After the blood-lactate curve has been plotted, a straight line is fitted from the two end points of the blood-lactate curve. The intensity associated with the widest point between the blood lactate curve and the straight line denotes the T_{lac}. The D_{max}

method of identifying the T_{lac} has been reported to be highly correlated ($r = 0.86$, $p < 0.001$) with running performance in both males and females (Nicholson & Sleivert, 2000).

Training at the $T_{lac,}$ or close to it, has been suggested to provide the optimum stimulus to enhance the T_{lac} (Tanaka, Watanabe, Konishi et al., 1986). However, further experimental evidence to support this is sparse (Midgley, McNaughton & Jones, 2007). In the study by Tanaka et al. (1986) the subjects were middle distance runners who typically performed high intensity training as part of their periodised training programme. Therefore, the change in training load (duration and intensity) may be the reason why the T_{lac} was enhanced with the inclusion of sub-maximal endurance training. In subjects already accustomed to longer sub-maximal runs no alteration in the T_{lac} is observed following further increases in training volume at the intensity corresponding to the T_{lac} (Londeree, 1997; Weltman, Seip, Snead, et al., 1992). An alternative approach to enhancing the T_{lac} involves the ingestion of certain supplements prior to exercise. Edge, Bishop and Goodman (2006) reported a significant improvement in the T_{lac} of 16 untrained women following eight weeks sodium bicarbonate ($NaHCO_3$) ingestion whilst undertaking a high-intensity interval training programme. Both the placebo group (training alone) and the $NaHCO_3$ group displayed improvements in the T_{lac} (15% vs 26%, $p \leq 0.05$); however, the improvements in the $NaHCO_3$ group were significantly greater ($p \leq 0.05$). Other evidence suggests short term metabolic alkalosis, via $NaHCO_3$ ingestion, can influence $\dot{V}O_2$ kinetics during a GXT (Zoladz, Szkutnik, Duda, Majerczak & Korzeniewski, 2005). Another buffering agent under recent investigation is beta alanine (β-alanine).

2.4 Carnosine

Beta alanine (β-alanine) is the rate-limiting precursor for the biosynthesis of the intramuscular buffer carnosine (Bate-Smith, 1938). Carnosine is a dipetide synthesised in skeletal muscle and brain tissue (Bakardjiev & Bauer, 1994; Bauer & Schulz, 1994) from the essential amino acid histidine and the non-essential β-amino acid β-alanine. This is catalysed by the enzyme carnosine synthetase (Kalyankar & Meister, 1959). Carnosine was originally discovered by a Russian scientist at the turn of the 20th Century (as cited by Boldyrev, 2007) but it was not until several decades later that its role in mammals as a muscular buffer was identified (Bate-Smith, 1938). More recently, research has elucidated that carnosine could participate in other important physiological roles, such as an antioxidant, an enzyme regulator and enhancing sarcoplasmic reticulum calcium release (Begum, Cunliffe & Leveritt, 2005). Evidence of carnosines primary role as an intramuscular buffer can be found from studies with different species (Abe, 2000). Certain species, who rely heavily on anaerobic energy production (racing dogs, horses), typically possess greater concentrations of intramuscular carnosine than species which are less active, for example humans (Harris, Marlin, Dunnett, Snow & Hultman, 1990). Additionally, higher concentrations of carnosine are reported in species which are exposed to extended periods of hypoxia (Davey, 1960). Little piked whales, for example, are capable of prolonged breath-hold dives and possesses a high concentration of HCD due to the hypoxic conditions it frequently inhabits (Abe, 2000).

Human carnosine concentrations show large inter-individual variation. Factors influencing concentrations include gender, age, training status, diet and β-alanine supplementation. Mannion et al. (1992) demonstrated that untrained males have significantly greater levels of carnosine in the quadriceps femoris compared to

females (21.3 ± 4.2μmol·g^{-1}dw vs 17.5 ± 4.8 μmol·g^{-1}dw, $p < 0.05$). As with species whose environment and activity permits long periods of hypoxia or acidosis, it appears that individual fibre types in humans demonstrate variation in carnosine content depending on the activity they are predominantly recruited for. Fibres which are primarily glycolytic (type II) have greater levels of carnosine than that of fibres that are recruited for oxidative energy provision. Harris, Dunnett and Greenhaff (1998) took muscle biopsies from the vastus lateralis of four subjects and stained them to determine the fibre type and determined carnosine content of each fibre by liquid chromatography. The results indicate that type II fibres have significantly greater carnosine levels compared to type I fibres (23.2 mmol·kg^{-1}dw vs 10.5 mmol·kg^{-1}dw, $p \leq 0.05$). Similar results were previously reported in the leg muscle of horses (Sewell,, Harris, Marlin & Dunnett, 1992). Collectively, these results further reinforce the primary role of carnosine as an intramuscular buffer as type II fibres predominantly replenish ATP via anaerobic glycolysis.

It may, therefore, seem logical that repetitive high-intensity training (i.e. training that would tax the anaerobic system) would inevitably augment muscle carnosine levels due to the greater recruitment of type II fibres during this type of activity (Linnamo, Newton, Hakkinen et al., 2000). Interestingly, a recent investigation (Tallon, Harris, Boobis, Fallowfield & Wise, 2005) reported that well trained bodybuilders have over double the amount of carnosine than that of their age-matched counterparts (43.01 ± 8.27 vs. 19.75 ± 3.31 mmol·kg^{-1}dry weight, $p \leq 0.001$). It was suggested that these levels of carnosine may be responsible for as much as 19% of the total muscle buffering capacity. In comparison, it was reported that in untrained individuals, the contribution of carnosine to total muscle buffering capacity is between seven and eight percent (Mannion, Jakeman, Dunnett, Harris & Willan,

1992; Tallon et al., 2005). However, the extent to which this is a purely training response cannot be easily identified from the data of Tallon et al (2005), as the two groups differed in many aspects besides their training regimes (e.g. fibre type proportion, diet, supplement usage, drugs etc). Nevertheless, comparisons between sprinters, rowers, marathon runners and untrained subjects support similar inquisitions regarding supramaximal training and improved carnosine concentrations (Parkhouse, McKenzie, Hochachka & Ovalle, 1985). The sprinters and rowers had significantly greater concentrations of carnosine compared to the marathon runners and the untrained individuals (4.93 ± 0.76 μmol·g^{-1}dw, 5.04 ± 0.72 μmol·g^{-1}dw vs 2.80 ± 0.74 μmol·g^{-1}dw, 3.75 ± 0.86 μmol·g^{-1}dw, $p \leq 0.01$). In a study assessing the effects of sprint training on muscle carnosine levels (Suzuki, Ito, Takahashi & Takamatsu, 2004), eight weeks of training was enough to exacerbate concentrations by more than double the preliminary amount. Collectively, this suggests that long term training may potentially enhance muscle carnosine concentration. However, the degree of improvement in carnosine concentrations is dependent on the type of training that is under taken.

Carnosine is not present in plants and, therefore, is absent from fruit and vegetables. As a result muscle carnosine concentrations of vegetarians maybe reduced. Indeed that was the case in a recent investigation assessing the variation in muscle carnosine content of vegetarians and omnivores (Harris, Jones, Hill, et al., 2007). Vegetarians were reported to have lower levels of carnosine in the vastus lateralis compared to those who ate a mixed diet (12.9 ± 2.8 mmol·kg^{-1}dm and 23.3 ± 5.4 mmol·kg^{-1}dm, respectively). Furthermore in another study, ingestion of chicken breast extract, which contains considerable amount of carnosine, was also reported to increase carnosine concentrations in male subjects (Sato, Suzuki, Morimatsu &

Takamatsu, 2003). Carnosine ingested from meat is hydrolysed, in the gastro-intestinal tract, into histidine and β-alanine by the enzyme carnosinase. The lack of this enzyme in muscle cells implies intramuscular carnosine is a stable compound (Otani, Okumura, Hashida-Okumura & Nagai, 2005). The two constituents are transported via the circulatory system to the brain and skeletal muscle, as these tissues possess the carnosine synthetase enzyme responsible for carnosine synthesis (Bauer & Schulz, 1994). The consequent uptake of β-alanine is dependent on its relative concentrations in the blood (Dunnett & Harris, 1999). Due to the greater affinity of carnosine synthetase for histidine, the rate limiting substrate for carnosine synthesis is therefore β-alanine (Artioli, Gualano, Smith, Stout & Lancha, 2010).

2.5 Beta alanine supplementation

β-alanine supplementation is, therefore, the most effective way to augment skeletal muscle carnosine concentrations (Harris, Tallon, Dunnett, et al., 2006). The degree of improvement in carnosine concentrations appears to be dose dependent with greater doses increasing carnosine levels to a greater extent. Table 2.1 gives a summary of the studies assessing carnosine increases following β-alanine supplementation. Harris et al. (2006) assessed dosages of 3.2 $g \cdot kg^{-1}d^{-1}$ and 6.4 $g \cdot kg^{-1}d^{-1}$ for a period of 28 days and reported that muscle carnosine levels had increased by 42 percent and 64 percent, respectively.

Table 2.1. Summary of β-alanine supplementation on muscle carnosine levels

Reference	Subjects	Dose	Carnosine increase	Protocol	Performance enhancement
Baguet et al., 2009	15 untrained males	4.8g·d^{-1} for 4-5weeks	Soleus 39%* Tibialis 27%* Gastrocnemius 23%* No ↑ in Pl group	N.A	N.A
Derave et al., 2007	15 sprint trained males	4.8g·d^{-1} for 4 weeks	Soleus 47% Gastrocnemius 37% No ↑ in Pl group	Matched-paired design. 5 x 30 isokinetic knee extensions, isometric contraction at 45% max, 400m run time	Bouts 4 & 5 isokinetic peak torque ↑* by 6.1 & 3.8%, respectively in β-Al group only.
Harris et al., 2006	21 physically active males	4weeks of: 1) 3.2g g·d^{-1} of β-Al 2) 6.4 g·d^{-1} of β-Al 3) 1000 – 2000g of carnosine 4) placebo	↑ in v.lateralis: 1) 42.1%* 2) 64.2%* 3) 65.8%* 4) no change		
Hill at al., 2007	25 physically active males	4 – 6.4 g·d^{-1} for 4 wks. 8 subjects continued to supple-ment for 10 weeks	↑ in v.lateralis by 58% and 80% for 4 and 10 wk, respectively. = ↑ in all fibres	Cycle ergometer test at 110% work rate max to assess TWD	13% and 16.2%↑* in TWD after 4 and 10 wk, respectively. No change in placebo group.

Table 2.1 continued…

Reference	Subjects	Dose	Carnosine increase	Protocol	Performance enhancement
Kendrick et al., 2009	7 recreationally active males	6.4g·d^{-1} for 4 weeks	↑ in v.lateralis of β-Al group only. Trained leg 52.2% untrained leg 28.3% = ↑ in all fibre types.	4 weeks of single-legged isokinetic training, with or without β-Al supplementation	N/A

*Note: * significant ($p < 0.05$), ↑ = increase, Pl – placebo group, β-Al = beta alanine group, v.lateralis = vastus lateralis, TWD = total work done.*

If the dosing period is extended from four weeks to ten weeks, it has been suggested that carnosine concentrations can be further augmented, by an additional 16 percent, when dosing with 6.4 g·kg^{-1}d^{-1} (Hill, Harris, Kim, et al., 2007). Other laboratories (Baguet, Reyngoudt, Poitter, et al., 2009; Derave, Özdemir, Harris, et al., 2007; Harris, Jones. Kim, et al., 2009; Kendrick, Kim, Harris, et al., 2009) have also demonstrated that muscle carnosine levels are increased following β-alanine supplementation for several weeks (Table 2.1). Alternatively, shorter dosing periods of 14 days have been employed (Harris, Hill, Sale, et al., 2006; Ponte, Harris, Hill et al., 2007). While muscle carnosine levels were not assessed in the aforementioned studies, performance was still improved using a 14 day supplementation protocol. While there seems to be little question of its effectiveness, there appears to be a noticeable inter-individual variation in muscular carnosine increases between subjects following β-alanine supplementation. Derave et al. (2007) reported increases of between 28 and 89 percent in the soleus and increases between 22 and 53 percent in the gastrocnemius of trained sprinters. This suggests that carnosine levels can be increased in trained athletes as well as untrained individuals when supplementing with β-alanine for four weeks. Although the average increase in carnosine levels

(gastrocnemius 37 percent) is slightly lower than those reported in other investigations (vastus lateralis 50 percent) using untrained subjects (Hill et al., 2007), it demonstrates that supplementation can augment carnosine levels in those with initially high levels of intramuscular carnosine. Albeit, more work is clearly warranted using highly trained subjects. The variation reported in the degree of carnosine improvement between the study of Derave et al. (2007) and Hill et al. (2007) could reflect the differences in the site where the muscle sample was extracted from. In another study, samples taken from the gastrocnemius muscle of 15 untrained subjects after four weeks β-alanine supplementation (Baguet et al., 2009), resulted in a 23 percent increase in carnosine concentrations. The contrasting findings of Derave et al. (2007) and Baguet et al. (2009) with regards to muscle carnosine increases may be related to the training regimes of the participants. The subjects of Derave et al. (2007) were all involved in sprint training of a relatively high standard as they were preparing for the indoor 400m season; the subjects from the Baguet et al. (2009) laboratory were reported as not being involved in regular training. It may, therefore, be a genetic factor as the sprinters could have a greater proportion of type II muscle fibres (Parkhouse et al., 1985), which have been reported to contain greater amounts of carnosine (Harris et al., 1998) and may result in a more rapid synthesis of carnosine than type I fibres. A recent investigation (Kendrick et al., 2009) assessed the differences in carnosine increases between type I and type II muscle fibres, following β-alanine supplementation. The results indicted that carnosine content was increased by 52, 35 and 65 percent in type I, type IIa and type IIb fibres, respectively. Interestingly, the greatest increase in carnosine was reported in the fibres where anaerobic energy delivery predominates, possibly due to enhanced carnosine synthetase activity (Tsubone, Yoshikawa, Okada & Abe, 2007) or/and enhanced β-

alanine transport (Bakardjiev & Bauer, 1994). This hypothesis has yet to be confirmed (Baguet et al., 2009).

2.6 β-alanine effects on exercise performance

Given the greater concentrations of carnosine in muscle fibres that predominantly produce ATP via anaerobic glycolysis, it would be coherent that β-alanine supplementation could theoretically enhance exercise performance in situations where a strong muscular metabolic acidosis is elicited, and thus becomes a limiting factor (i.e. 60 seconds to 5 minutes). Table 2.2 summaries the effect of β-alanine supplementation on performance indices.

Table 2.2 Summary of the effects of β-alanine supplementation on performance

Reference	Subjects	Dose	Carnosine increase	Protocol	Performance enhancement
Baguet et al., 2010	14 physically active students	4.8 $g \cdot d^{-1}$ for 4 wks	Not measured	MP design. 6 min cycling on ergometer @ 50% between VT and $\dot{V}O_{2max.}$ $\dot{V}O_2$ kinetics were recorded. Blood samples taken to assess pH, lactate, bicarbonate and base excess.	Exercise-induced acidosis was reduced* by 19% in β-Al group. No effect on the O_2 deficit.
Derave et al., 2007	15 sprint trained males	4.8$g \cdot d^{-1}$ for 4 wks	Soleus 47%* Gastroc - nemius 37%*	MP design. 5 x 30 isokinetic knee extensions, isometric contraction at 45% max, 400m run time	Bout 4 & 5 peak torque increased* by 6.1 & 3.8%, respectively in β-Al group only

Table 2.2 continued…

Reference	Subjects	Dose	Carnosine increase	Protocol	Performance enhancement
Hoffman et al., 2007	26 male strength/ power athletes	4.5g·d^{-1} for 30 days. 9 days during training	Not measured	30sec Wingate test & intermittent sprint test. Subjective feelings of fatigue, soreness and practice intensity. Training logs recorded.	Subjective feeling of fatigue lower* in β-Al group. No difference in any other measures between β-Al and Pl groups
Jordon et al., 2010	17 recreationally active males	6g·d^{-1} for 4 wks	Not measured	GXT on treadmill to assess if ·$O_{2max,}$ %·$O_{2max,}$ ·O_2, HR or %HR_{max} at OBLA were affected.	%·O_{2max}, HR and %HR_{max} were improved* following supplementation
Smith et al., 2009	46 untrained males	6g·d^{-1} for 3 wks 3g·d^{-1} for remaining 3 wks	Not measured	β-Al supp and interval training for 6 wks. ·$O_{2max,}$ TTE, VT and TWD assessed before, during and after.	Gains in ·$O_{2max,}$ TTE and TWD significantly* greater during final 3 wks of training.
Stout et al., 2007	22 untrained females	3.2 – 6.4 g·d^{-1} for 4 wks	Not measured	Continuous cycle ergometer test to assess PWC_{FT}, VT, TTE and ·O_{2max}	In β-Al group 13.9, 12.6 and 2.5% increase* in VT, PWC_{FT} and TTE, respectively. No effect on ·$O_{2max.}$

Table 2.2 continued…

Reference	Subjects	Dose	Carnosine increase	Protocol	Performance enhancement
Stout et al., 2006	51 untrained males	1) placebo 34g 2) CR 5.25g 3) β-Al 1.6g 4) β-Al + CR. Taken 4x a day for 6days then 2x a day for next 22 days.	Not measured	Continuous cycle ergometer test to assess PWC_{FT}	14.5% increase* in PWCFT in the β-Al group. 11% increase in CR group. No additional effects of combined β-Al and CR.
Stout et al., 2008	9 males, 17 females, both un-trained	2.4 $g \cdot d^{-1}$ for 90 days	Not measure	Discontinuous cycle ergometer test to assess $PWC_{FT.}$	28.6% increase* at PWC_{FT} in the β-Al group. No change in Pl
Sweeney et al., 2010	20 strength/ power trained males	4 $g \cdot d^{-1}$ for 5 wks	Not measured	2sets 5 x 5sec sprints 45sec rest between sprints. 2 min rest between sets.	No performance enhancement
Van Theinen et al., 2009	17 moderate – well trained cyclists	2 – 4 $g \cdot d^{-1}$ for 8 wks	Not measured	110min cycling at 50% and 90% MLSS. 110min TT	Significantly* greater peak (11.4%) and mean (5.0%) power output during TT

Table 2.2 continued…

Reference	Subjects	Dose	Carnosine increase	Protocol	Performance enhancement
Zoeller et al., 2007	55 untrained males	1) Pl 2) CR, 10.5 – 21 $g \cdot d^{-1}$ for 4 wks 3) β-Al, 3.2 - 6.4 $g \cdot d^{-1}$ for 4 wks 4) CR+βAl (same dose as 2 & 3)	Not measured	GXT on cycle ergometer to determine VO_{2peak}, VT &LT	1) no effect 2) increase* in power at VT 3) increase* in power at LT 4) increase* in $\dot{V}O_2$ and power at LT and VT, increase in $\%\dot{V}O_{2peak}$ at VT

*Note. * = significant ($p < 0.05$), MP = matched-paired, VT = ventilatory threshold, β-Al = beta alanine, Pl = placebo, GXT = graded exercise test, $\dot{V}O_{2max}$ = maximum oxygen uptake, HR = heart rate, HR_{max} = maximum heart rate, TTE = time to exhaustion, TWD = total work done, PWC_{FT} = physical working capacity at the fatigue threshold, MLSS = maximal lactate steady state, LT = lactate threshold.*

Derave et al. (2007) reported no significant improvement in 400 metre sprint time for a group of 15 well-trained 400 metre runners, following four weeks β-alanine supplementation (pre: 51.11 ± 1.66 seconds vs. post: 50.36 ± 1.43 seconds, $p > 0.05$). Although not statistically significant, the improvement of 0.35 seconds should be viewed in light of the bigger picture. In high-level athletic competition even an enhancement of 0.3 to 0.4 of the coefficient of variation would prove to be an improvement in top level athletic competition (Hopkins, Hawley & Burke, 1999). It could be concluded that 400 metre sprint performance is not limited by intramuscular buffering capacity in this population of athletes. In the same group of athletes, peak torque was assessed via 5 bouts of 30 maximal knee extensions. During the last two bouts peak torque was significantly improved in the β-alanine group following supplementation (bout 4: +6.1% and bout 5: +3.8%, $p < 0.05$), compared to the placebo group (bout 4: + 1.0% and bout 5: -0.8%, $p > 0.05$). These results suggest that

β-alanine supplementation can attenuate fatigue during repeated maximal contractions, possibly due to an enhanced intracellular buffer capacity. In contrast, other authors employing intermittent type exercise protocols have reported less convincing findings. Sweeney, Wright, Brice & Doberstein (2010) using 20 power/strength trained males, employed a double-blind, placebo-controlled study using a matched paired design, to assess the effects of five weeks β-alanine supplementation ($4g \cdot d^{-1}$ in first week $6.4g \cdot d^{-1}$ four weeks) on fatigue percentage, horizontal peak and mean power during repeat sprints. Subjects performed two sets of five, five-second sprints, with 45 seconds rest between repetitions, separated by two minutes rest between sets. No significant differences ($p > 0.05$) were reported for group, time or the interaction of time x group for peak power, mean power or fatigue percentage. A possible reason for the lack of significant findings could stem from the protocol employed. It could be questioned whether multiple five-second sprints, with a 45 second rest period between, would tax the anaerobic glycolytic system sufficiently to elicit metabolic acidosis (Glaister, 2005). It was reported previously (Gaitanos, Williams, Boobis & Brooks, 1993) in a similar protocol to that employed by Sweeney et al. (2010) that the percentage of energy derived from anaerobic glycolysis was considerably reduced during the final sprints. Furthermore, no lactate accumulation was apparent during the final sprint and the author proposes that the energy for the final sprint was derived from phosphocreatine and aerobic metabolism.

Studies assessing the impact of β-alanine supplementation on maximal strength have been reviewed in several recent publications (Artioli et al., 2010; Culbertson, Kreider, Greenwood & Cooke, 2010; Derave, Everaert, Beeckman & Baguet, 2010; Wilson, Wilson, Zourdos, Smith & Stout, 2010). As maximal strength has little, if any, effect on endurance performance, the effects of β-alanine

supplementation on maximal strength are deemed irrelevant here and will not be discussed. The influence of β-alanine supplementation on aerobic parameters yields some discrepant findings. In one study (Harris et al., 2006), isometric endurance of the knee extensors at 45 to 50 percent maximum voluntary contraction (MVC) was improved by 11.4% ($p < 0.02$) following 14 days β-alanine supplementation (6.4 $g \cdot d^{-1}$). Likewise, Ponte, Harris, Hill et al. (2006) reported an 11.1 percent increase in isometric endurance of the knee extensors at 45 to 50 percent MVC following a 28 day supplementation period (6.4 $g \cdot d^{-1}$). The similar increases in muscle endurance reported by Harris et al. (2006) and Ponte et al. (2007) following the two different supplementation periods suggests that muscle carnosine can be enhanced and directly attenuate fatigue by using a shorter dosing period without the subsequent ergogenic effect being attenuated. It is deduced, therefore, that muscular carnosine is augmented by similar amounts with a shorter supplementation period, yet to what extent still needs to be clarified.

Hill et al (2007) reported increases in muscle carnosine concentrations of 58.8 percent and 80.1 percent with a four and ten week supplementation period, respectively. This lead to a 13 percent and 16.2 percent increase ($p < 0.05$) in total work done (TWD) in a cycling capacity test at 110 percent of work rate maximum (WRM). In a recent study (Van Theinen, Van Proeyen, Vanden Eynde, et al., 2009), subsequent to eight weeks of β-alanine supplementation (2- 4 $g \cdot d^{-1}$), mean and peak power outputs were improved in a ten minute cycle performance test following a two hour endurance cycle. Mean power was enhanced by 5 percent and peak power by 11.4 percent ($p < 0.05$) yet there were no differences in lactate and pH levels which suggests that the increased buffering capacity enabled subjects to work at a higher intensity for the ten minute performance test. Enigmatically, during the two hour

endurance bout prior to the sprint performance test, there were no differences in lactate or pH between the β-alanine and placebo group, which suggests that sub-maximal exercise is unaffected by β-alanine supplementation. In a recent study, TWD was enhanced following a combined β-alanine supplementation and high-intensity training programme (Smith, Walter, Graef et al., 2009). Forty-six males undertook a GXT to determine $\dot{V}O_{2max}$, time to exhaustion, ventilatory threshold and TWD. Subjects were then randomly assigned to a supplement group ingesting either $6g \cdot d^{-1}$ of β-alanine or a placebo during six weeks of high-intensity interval training on a cycle ergometer. Supplementation continued at a lower absolute dose ($3g \cdot d^{-1}$) after the first 21 days. Parameters were measured before, after the first three-weeks, and then upon completion of the six week training period. There was a significant ($p < 0.05$) improvement in $\dot{V}O_{2max,}$ time to exhaustion and TWD in both groups, following the six week training programme. The gains in $\dot{V}O_{2max}$ (4.26%, $p = 0.010$) and time to exhaustion (2.80%, $p = 0.043$) during the three to six week period were only significant in the β-alanine group. Other studies assessing the impact of β-alanine supplementation on $\dot{V}O_{2max}$, however, have failed to find any improvements following supplementation (Hill et al., 2007; Zoeller, Stout, O'Kroy, Torok & Miekle, 2007), suggesting that the results reported by Smith et al. (2009) are merely a result of the training programme rather than the supplement *per se*. Nevertheless, β-alanine supplementation may be a valuable training aid. Hoffman, Ratamess, Faignbaum et al. (2007) examined the effects of 30 days β-alanine supplementation in 26 collegiate American football players. For the final nine days of supplementation subjects began pre-season training and under went a Wingate test and sprint drills test. Training logs were recorded and after training sessions subjects completed questionnaires on subjective feelings of soreness, fatigue, and practice intensity. There were no

significant differences ($p > 0.0.5$) between the β-alanine and placebo group for the Wingate test, the sprint drills, feelings of soreness or perception of practice intensity. Subjects reported significantly lower feelings of fatigue in the β-alanine group compared to the placebo group (3.96 ± 0.80 vs. 4.55 ± 0.83 on a 1 to 7 scale, $p < 0.05$). There was also a significant increase ($p < 0.05$) in the training volume during bench press exercise in the β-alanine group. This suggests that β-alanine supplementation can increase the amount of work done during training, by delaying fatigue and reducing subjective feelings of fatigue.

Besides enhancing TWD, time to exhaustion and power during aerobic exercise, studies assessing the effect of β-alanine supplementation on neuromuscular fatigue have also reported promising results, in young (Stout, Cramer, Meilker, et al., 2006; Stout, Cramer, Zoeller et al., 2007) and ageing populations (Stout, Graves, Smith et al., 2008). A test labelled the physical working capacity at fatigue threshold (PWC_{FT}), uses the electromyographic (EMG) amplitude to detect the power output that corresponds to neuromuscular fatigue during incremental cycle exercise. Stout et al. (2006) recruited 51 untrained males to assess the effects of four weeks β-alanine supplementation ($6.4g \cdot d^{-1}$) on neuromuscular fatigue of the vastus lateralis during submaximal cycling at the PWC_{FT}. Beta alanine supplementation resulted in a 14.5 percent improvement ($p < 0.01$) in the PWC_{FT} compared with pre-supplementation values. There was no change reported in a placebo group. Furthermore, combining β-alanine supplementation with creatine produced no additive effects (11 percent increase in PWC_{FT}, $p < 0.05$). Stout et al. (2006) concluded that the increase observed in PWC_{FT} following β-alanine supplementation was a result of augmented skeletal muscle carnosine concentrations thereby enhancing the buffering capacity during exercise. Although men possess a greater skeletal muscle carnosine concentration

(Mannion et al., 1992) and a superior anaerobic exercise capacity (Mannion, Jakeman & Willan, 1995) it appears the ergogenic effects of β-alanine supplementation are not restricted to the male sex. In 22 untrained females four weeks β-alanine supplementation (3.2 – 6.4g·d^{-1}), enhanced the PWC_{FT} (12.6 percent, $p < 0.001$), the ventilatory threshold (13.9 percent, $p < 0.0.01$) and the time to exhaustion (2.5 percent, $p < 0.05$) during GXT (Stout et al., 2007).

The ventilatory threshold is defined as the non-linear rise in ventilation with ·O_2 during a GXT, and is thought to relate to the increased production of carbon dioxide caused by the buffering of H^+ as it accumulates in the blood during exercise (Stout et al., 2006). It has been suggested that the T_{lac} and the ventilatory threshold occur at a similar point when graphically represented (Bearver, Wasserman & Whipp, 1986). Therefore, many believe there is a close association between the two parameters. Beta-alanine supplementation reportedly increased the ventilatory threshold in untrained women by 13 percent compared to a placebo group (Stout et al., 2007). In contrast, the ventilatory threshold in young men (22.2 ± 3.3 years) was not further enhanced by β-alanine supplementation combined with high intensity training, when compared to a training only group (Smith, Walter, Kendall, et al., 2008). More recently, Smith et al. (2009) assessed the combined effect of six weeks interval training and β-alanine supplementation (3-6g·d^{-1}) on the ventilatory threshold, and several other physiological parameters during a GXT. In agreement with the previous research (Smith et al., 2008; Zoeller et al., 2007) there was no improvement in the ventilatory threshold other than that imposed by the interval training. Another four week study (Zoeller et al., 2007), that assessed the effects of β-alanine (6.4g·d^{-1}) and creatine (21g·d^{-1}), alone or in combination, reported that β-alanine alone did not have a significant ($p > 0.05$) effect on ·O_{2peak}, ·O_2 at the T_{lac} or ventilatory threshold.

However, ·O_2 and power output at the T_{lac}, and ventilatory threshold, were significantly ($p < 0.05$) improved when β-alanine and creatine supplementation were combined. The authors concluded that the increase in power at the T_{lac} with β-alanine supplementation could be due to an augmented H^+ buffering capacity in skeletal muscle due to increases in carnosine concentrations. In agreement with Zoeller et al. (2007) a recent investigation reported no effect of β-alanine supplementation on ·O_2 kinetics. Fourteen physically active male subjects were randomised into two groups. One group supplemented with β-alanine ($4.8g \cdot d^{-1}$) and the other with an identical amount of a placebo for four weeks. Subjects performed a six minute cycle ergometer test before and after the supplementation period while ·O_2 kinetics were recorded and blood samples were extracted to assess pH, lactate, bicarbonate and base excess. The results indicate that there were no significant ($p < 0.05$) effect on ·O_2 kinetics following β-alanine ingestion. On the contrary, the exercise-induced increase in pH was reduced by 19 percent compared to the placebo group ($p = 0.03$). In a different study, 17 recreationally active men undertook a GXT prior to four weeks β-alanine supplementation ($6g \cdot d^{-1}$) to assess if heart rate, percentage of heart rate maximum at the onset of blood lactate (OBLA), percentage ·O_{2max} at OBLA and ·O_{2max} were altered following supplementation (Jordon, Lukaszuk, Misic & Umoren, 2010). The percentage of heart rate maximum at OBLA and percentage ·O_{2max} at OBLA were significantly increased (5.6 and 6.5 percent, respectively; $p < 0.05$) following β-alanine ingestion. However, the author reported a significant decrease in □O_{2max} in both relative and absolute terms (-6.3 and -5.7 percent, respectively; $p < 0.01$) after β-alanine supplementation. The greater reduction in □O_{2max} in relative terms is due to an increase in body mass of the β-alanine group. The reduction in □O_{2max} weakens some of the findings from the investigation. For instance, the enhancement of the

percentage □O_{2max} at OBLA may not be a true reflection of physiological response to the β-alanine supplementation and may merely be a reflection of the decrease in □O_{2max} and should be interpreted with caution. Secondly, the protocol used to identify OBLA was a fixed blood lactate concentration of 4mmol·L^{-1}. Using a fixed blood lactate concentration may make identification purposes easier but it does not take into account inter-individual variation in blood lactate response nor does it enable the use of the T_{lac} as a flexion point (Faude, Kindermann & Meyer, 2009; Binder, Wonisch, Coora, et al., 2008).

To summarise, intramuscular carnosine acts as a buffer during high-intensity exercise in humans. Individual carnosine concentrations are affected by several factors, including gender, training status, the proportion of fast-twitch muscle fibres and β-alanine supplementation. Four-to-ten weeks β-alanine supplementation has the potential to enhance muscle carnosine concentrations by up to 80 percent. Supplementation can increase carnosine levels in both athletic and non-athletic populations alike. The ergogenic effects of β-alanine supplementation are evident in exercise situations whereby anaerobic energy provision predominates. Single and multiple bouts of short-duration, high-intensity exercise are improved following β-alanine supplementation. Additionally, research suggests that β-alanine supplementation can exert an ergogenic effect in exercise scenarios in which the intensity is below ·O_{2max}.

Therefore, the purpose of this study was to assess, using a double-blind, placebo-controlled, repeated measures design, if β-alanine supplementation can improve the velocity and the percentage of ·O_{2max} at the T_{lac} during treadmill running. In addition, the·O_2, blood lactate, HR and RPE at the T_{lac} will also be assessed following β-alanine supplementation as these parameters have been reported to be

affected by supplementation (Baguet et al., 2010; Hoffman et al., 2007; Zoeller et al., 2007). The D_{max} method of measuring the T_{lac} was preferred to a fixed lactate concentration to enable individual response to be identified. As aforementioned, previous research has reported the effectiveness of augmenting muscle carnosine concentrations by supplementing with β-alanine for a period of four to ten weeks. The ergogenic effects of β-alanine have been reported after supplementing for only two weeks. In order to make supplementation with β-alanine more practical the present investigation will assess if a five day supplementation period can produce an ergogenic effect.

2.7 Hypotheses

Primary hypothesis: There will be a significant difference in the running velocity at the T_{lac} between the control, placebo and β-alanine trials following five days β-alanine supplementation ($50mg \cdot kg^{-1} \cdot d^{-1}$). Previous research reported that β-alanine supplementation increased the power at the T_{lac} during cycle exercise in untrained subjects (Zoeller et al., 2007). It is proposed, therefore, that β-alanine will significant improve the velocity at the T_{lac} during treadmill running.

Secondary hypotheses: There will be a significant difference between the control, β-alanine and placebo trials, in the percentage of $\dot{V}O_{2max}$ at which the T_{lac} occurs, following five days β-alanine supplementation. This will be in conjunction with a significant difference in the $\dot{V}O_2$ at the T_{lac} between the three trials. Beta-alanine supplementation was recently reported to enhance the percentage of $\dot{V}O_{2max}$ at a fixed blood lactate concentration in recreationally active subjects (Jordon et al., 2010). Furthermore, there will a significant difference in the lactate, HR and RPE response at the T_{lac} between the control, β-alanine and placebo trials. It has been suggested that β-

alanine supplementation can alter blood lactate response (Baguet et al., 2010), HR (Jordon et al., 2010) and RPE (Hoffman et al., 2007). Therefore, these parameters were monitored to assess if β-alanine supplementation could alter the response of these factors at the T_{lac}.

3. *Method*

3.1 Participants

A group of 6 (4 males, 2 females) healthy, recreationally active individuals participated in the study. Participants' anthropometric data is displayed in table 3.1. Participants inclusion criteria were: 1) aged < 45 years, 2) being free from illness, disease or injury for a month prior to the start in of the study, 3) having a resting heart rate and blood pressure < 90 bpm and 160/100mmHg, respectively, 4) not currently taking any nutritional supplements, 5) being a non-smoker, 6) being habitually active and accustomed to physical exertion. Exclusion criteria included: 1) aged > 45 years, 2) having a current illness or injury within the last month, 3) having a resting heart rate and blood pressure > 90 bpm and 160/100mmHg, respectively, 4) currently taking any nutritional supplements, 5) having been a regular smoker in the past, 6) being habitually inactive and unaccustomed to physical exertion. All were informed of the purpose of the study and associated risks prior to signing consent forms (Appendix A) and taking part. A risk assessment was compiled before the onset of testing and health questionnaires (appendix B) were completed prior to each testing session. The protocol was approved by the University of Chester Ethics Committee.

Table 3.1 Subjects descriptive characteristics (n=6).

	Age (years)	Weight (kg)	Height (cm)	BMI (kg/m^2)	$\dot{V}O_{2max}$ ($ml \cdot kg^{-1} \cdot min^{-1}$)	$\dot{V}O_{2max}$ ($L \cdot min^{-1}$)
Mean	27.83	68.43	172.52	22.80	53.26	3.66
SD	2.48	10.85	9.59	1.36	7.81	0.83

Note. M = mean; *SD* = standard deviation; BMI = body mass index; $\dot{V}O_{2max}$ = maximum oxygen uptake

3.2 Experimental Design

This double-blind, placebo-controlled, repeated measures experimental design (figure 3.2) was chosen over the more popular matched paired design due to the stronger statistical within-subjects comparison (Atkinson & Nevill, 2001; Winter, Eston & Lamb, 2001). Participants were asked to refrain from any exhaustive exercise and from consuming alcohol and caffeine in the 48 hours prior to each testing session. This was assessed via verbal confirmation from the individual prior to testing. Participants were instructed to keep to their usual dietary and exercise regimes and replicate them in the 48 hours prior to each trial. Additionally, participants were instructed to refrain from exercising on the day of testing. This was assessed via verbal confirmation from the participant. It has been suggested that diet has little influence on muscle carnosine levels (Baguet et al., 2009). Therefore, participants were not asked to record their food intake. Following the first visit to the lab for $\dot{V}O_{2max}$ assessment, trial order (control, placebo and β-alanine) was randomly assigned for the participants remaining three visits. Following the placebo and β-alanine trials a three week washout period was incorporated to allow for any supplement-induced carnosine increase to be hydrolysed and eliminated. With a typical supplementation period of 28 days, carnosine increases are reported to be augmented by about 42 percent (Harris et al., 2006). It is proposed the five-day supplementation period employed in the current study has the potential to increase carnosine concentrations by 7 to 10 percent. With the elimination of carnosine occurring at about four percent per week (Baguet et al., 2009), a three week washout period would be sufficient to return carnosine concentrations to pre – supplementation levels. Testing took place between the hours of 10am and 6pm. With the exception of one individual,

participants were tested at the same time of day for all trials in order to eliminate any effects due to circadian rhythms (Atkinson & Reilly, 1996).

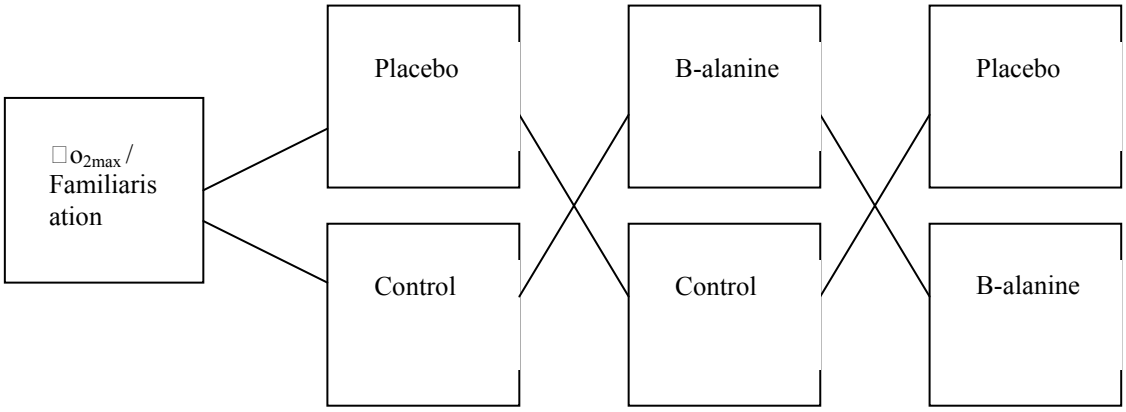

Figure 3.2. Diagrammatic representation of the double-blind, placebo-controlled, repeated measures cross-over experimental design (n = 6).

3.2.1 ▪O2max

On the first of four visits to the Human Performance Laboratory participants undertook a maximal treadmill (HP Cosmos, Pulsar, Nussdorf, Germany) test to assess ▪O_{2max} and familiarise participants to laboratory procedures. Anthropometric data was collected barefoot and in light weight clothing. Respiratory gases were assessed breath by breath via open-circuit spirometry (Cosmos, Quark, b2, Cosmed S.r.l, Rome, Italy). The Quark b2 was calibrated prior to each session using a 3 litre syringe for flow volumes across a wide range of flow rates and known gases for CO_2 and O_2 concentrations. Heart rate (HR) was continuoulsy monitored using FSI Polar Heart Rate monitors (Polar Electro Oy, Kempele, Finland). RPE was taken during the

last 30 seconds of each 3 minute stage (appendix C) using Borg's 6 – 20 scale (Borg, 1998). The test was preceded by a 5 minute warm-up during which the intensity was selected by the participant and replicated for subsequent trials. The face mask (Hans Rudolf, Kansas City, USA) used to collect respiratory gases was fitted following the warm-up. After which, the treadmill gradient was increased to one percent to replicate the energy costs of outdoor running (Jones & Doust, 1996) and a starting speed of 7 $km \cdot h^{-1}$ was selected. The speed was increased by 1 $km \cdot h^{-1}$ every 3 minutes until volitional exhaustion. The data for the last minute of each 3 minute stage was averaged over the final 30 seconds. The highest 30 second average for $\dot{V}O_2$ was recorded as the $\dot{V}O_{2max}$. Criteria for the attainment of $\dot{V}O_{2max}$ were two or more of the following: 1) no increase in HR with an increase in exercise intensity, 2) a respiratory exchanged ratio of >1.1, 3) a lactate concentration of > 8mM, 4) an RPE of > 17 on the on the Borg scale and 5) a plateau in $\dot{V}O_2$ (Whaley et al, 2006).

3.2.2 Lactate threshold

On remaining visits to the Human Performance Laboratory participants were assessed for T_{lac} and the $\dot{V}O_2$ at T_{lac} using a discontinuous 3 minute stage treadmill protocol. On arrival to the laboratory a finger tip blood sample was taken and analysed immediately for lactate using a portable lactate analyser (Lactate Pro, Arkray Inc, Kyoto, Japan). A puncture site (index or middle finger) was selected and cleaned with an alcohol pad. The area was allowed to dry and then wiped with a cotton pad to remove any residue alcohol, to prevent the blood from becoming haemolysed (Maw, Locke, Cowley & Witt, 2000). The sampling finger was held firmly and punctured into the pulp and across the finger using a single-use lancet. The first drops of blood were wiped away with a cotton pad as it may have been contaminated with other

bodily fluids. Moderate pressure was applied to the finger to ensure adequate blood flow and the sample was collect in Lactate Pro strips (Arkray Inc, Kyoto, Japan) and analysed by the Lactate Pro analyser. The protocol was the same as that described for the assessment of $\dot{V}O_{2max}$, except that after completion of each stage, a fingertip blood sample was taken (as described above) as the participant straddled the moving treadmill belt. The blood sampling procedure took between 20 and 40 seconds and then the participant resumed running at the next intensity. The T_{lac} was calculated via the D_{max} method (Cheung et al., 1992). Individual graphs were plotted, with treadmill speed ($km \cdot h^{-1}$) on the *x* axis and lactate concentration ($mmol \cdot L^{-1}$) on the *y* axis (figure 3.3). A straight line was then fitted between the two end lactate points and the maximal perpendicular distance from the fitted line was taken as the T_{lac}. $\dot{V}O_2$ at the T_{lac} was calculated from the same graph by the addition of a secondary *y* axis. The $\dot{V}O_2$ that corresponded to the T_{lac} was taken as the $\dot{V}O_2$ at the T_{lac}. For example, in figure 4.2 the velocity and $\dot{V}O_2$ at the T_{lac} corresponds to $12km \cdot h^{-1}$ and $37.71ml \cdot kg^{-1} \cdot min^{-1}$, respectively. This was done for all participants across all three trials. Appendix D shows an example from one for the participants. HR at T_{lac} was taken as the average HR over the last 30 seconds of the velocity corresponding to the T_{lac}.

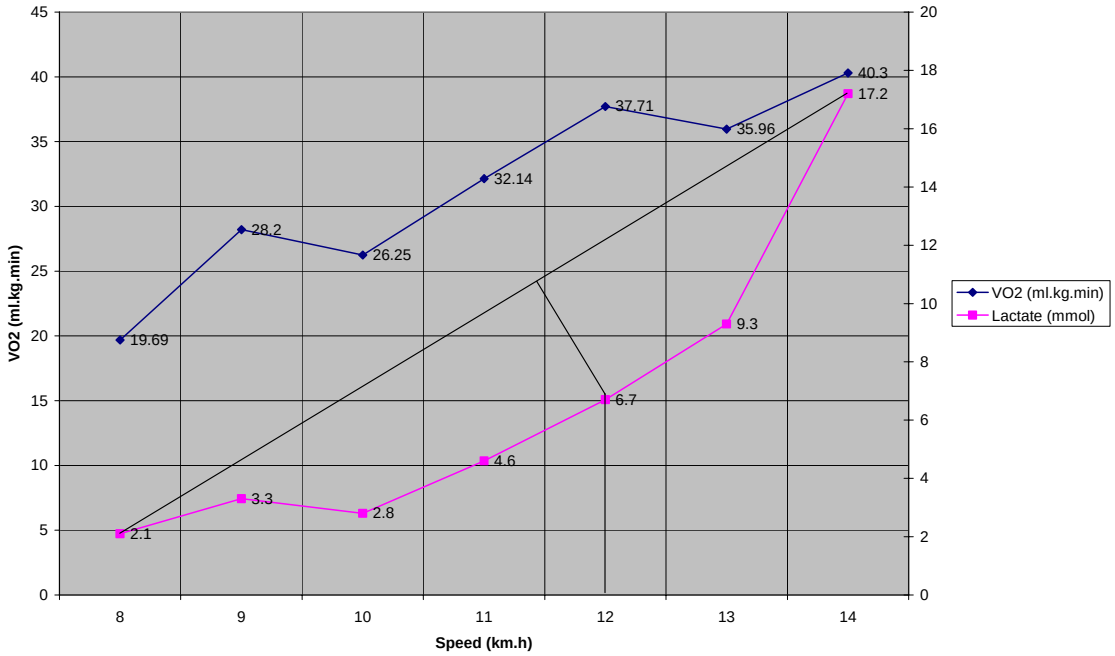

Figure 3.3. Lactate threshold graph depicting the velocity and $\dot{V}O_2$ at the at the lactate threshold calculated via the D_{max} method (Cheung et al., 1992).

3.2.3 Supplementation.

During the course of the study participants were asked to refrain from taking any other nutritional supplements. Participants were instructed to take 50 $mg \cdot kg^{-1} \cdot d^{-1}$ of β-alanine, or an equal amount of placebo (maltodextrin), for five consecutive days. This was based on the observations and recommendation of Hoffman et al. (2007) whose work suggests that participants should be given doses relative to body weight. Following the familiarisation/$\dot{V}O_{2max}$ session, the 50 $mg \cdot kg^{-1} \cdot d^{-1}$ of β-alanine and placebo were weighed out, according to each participant's weight, and put into gelatine capsules. The capsules were then separated into five envelopes for each

participant, with each envelope containing one days worth of β-alanine or the placebo. Participants were instructed to take no more than two capsules (≤ 800g) in any one dose and disperse the supplements equally throughout the day, with at least three hours between separate doses. This was in order to attempt to keep the participants blind to the substance they were ingesting as doses in excess of 800g of β-alanine have been reported to cause paresthesia (Harris et al., 2006). The supplements were identical in taste and appearance. A certificate of analysis for the β-alanine can be found in appendix E and confirms that it was 100 percent pure β-alanine.

3.2.4 Statistical analysis

The data met criteria for normal distribution. Data are, therefore, presented as means plus or minus the standard deviation (±SD). A repeated-measures analysis of variance (ANOVA) was performed to detect within-subject differences between the three trials (control, placebo and β-alanine), for the velocity, the $\dot{V}O_2$, the percentage of $\dot{V}O_{2max}$, the lactate concentrations, the HR and the RPE at T_{lac}. All data met the assumptions for sphericity ($p > 0.05$). An α level of $p < 0.05$ was chosen to indicate statistical significance. Data was analysed using SPSS for Windows (version 17.0, 2008, Chicago, IL).

4. Results

Participants ingested β-alanine or a placebo for five days prior to undertaking a T_{lac} test. A control trial was also performed where participants undertook a T_{lac} test without ingesting any ergogenic substances. The velocity, percentage $\dot{V}O_{2max}$, $\dot{V}O_2$, lactate, HR and RPE at the T_{lac} were measured for all trials. Table 4.1 displays the mean (±SD) for the velocity, percentage $\dot{V}O_{2max}$, $\dot{V}O_2$, HR and RPE at the T_{lac} for all three trials. One of the participants contracted the influenza virus prior to one of the testing sessions and therefore the control results were calculated with the data from 5 participants.

Table 4.1. Mean (± S.D.) for Velocity, percentage of $\dot{V}O_{2max}$, $\dot{V}O_2$, heart rate and RPE at the lactate threshold for all three trials

	Trial		
	Control (n = 5)	B-alanine (n = 6)	Placebo (n = 6)
Velocity at T_{lac} ($km \cdot h^{-1}$)	10.83 ± 1.16	10.17 ± 0.98	10.33 ± 1.03
%$\dot{V}O_{2max}$ at T_{lac} (%)	69.94 ± 7.39	75.21 ± 6.84	75.93 ± 7.32
$\dot{V}O_2$ at T_{lac} ($ml \cdot kg^{-1} \cdot min^{-1}$)	37.16 ± 5.68	40.21 ± 7.63	40.38 ± 6.29
Lactate at T_{lac} ($mmol \cdot L^{-1}$)	4.0 ± 1.8	4.1 ± 1.2	3.7 ± 1.5
HR at T_{lac} (BPM)	168.8 ± 13.9	165.6 ± 16.3	166.4 ± 11.6
RPE at T_{lac}	14.4 ± 1.7	14.0 ± 2.5	13.6 ± 2.3

Table 4.1 gives an overview of the measured parameters. The velocity at the T_{lac} was reduced during the β-alanine and placebo trials. The $\dot{V}O_2$ at T_{lac} was increased following β-alanine supplementation and this was also true during the placebo trial. Therefore, percentage of $\dot{V}O_{2max}$ at T_{lac} also increased during the β-alanine and

placebo trials. Lactate values compared to the control trial were increased in the β-alanine trial and attenuated in the placebo trial. HR and RPE response was reduced in the treatment trials compared to the control. The results for each parameter measured are presented in greater depth below.

4.1 Body mass

Participants mean body mass during the testing period remained relatively stable. Mean body mass at the beginning of testing was not significantly different to that recorded at the end of the testing period (68.43 ± 10.85 vs. 68.81 ± 11.45 kg, p = 0.497). Changes in $\dot{V}O_2$ kinetics, therefore, reflect response to treatments rather than fluctuations in body mass.

4.*2 Velocity at the lactate threshold*

Mean ± SD for the velocity at the T_{lac} was 10.83 ± 1.16, 10.17 ± 0.98 and 10.33 ± 1.03km·h^{-1} for the control, β-alanine and the placebo trial, respectively (figure 4.2). The velocity at the T_{lac} was reduced by 4.6% in the placebo trial, and reduced by an additional 1.8% in the β-alanine trial, when compared to the control trial. A repeated measures ANOVA revealed that there was no significant differences between the three trials (p = 0.369).

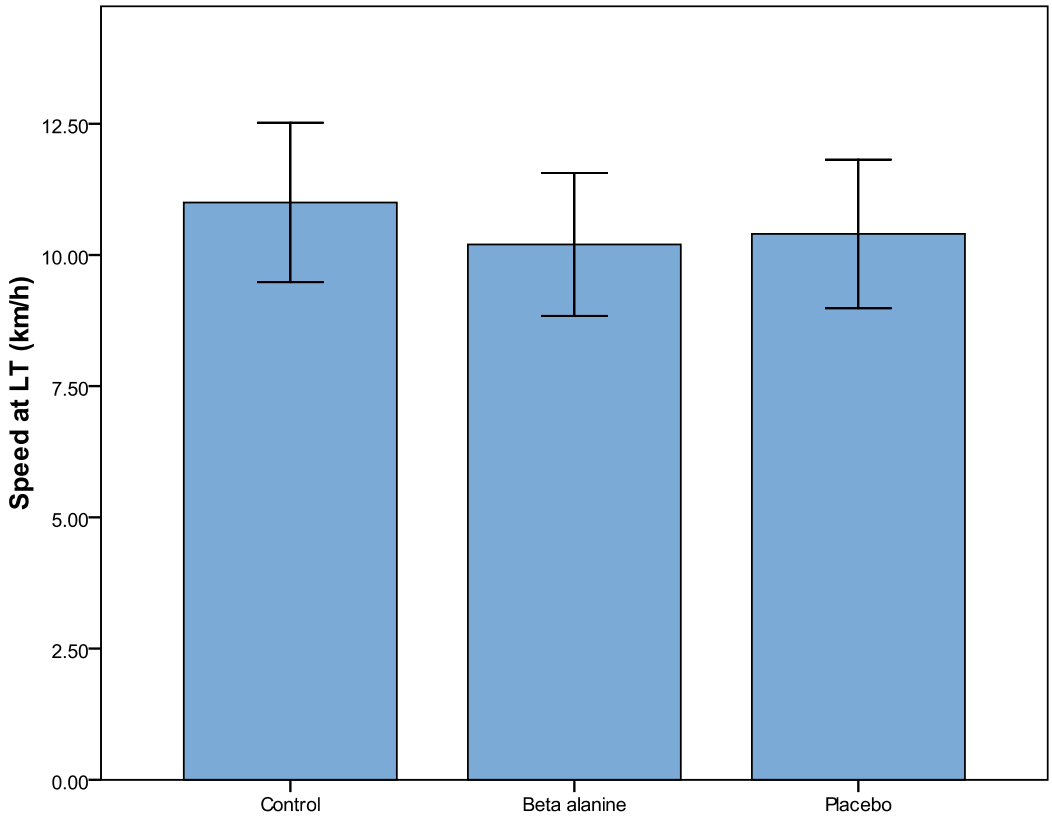

Figure 4.2 Mean ± (SD) velocity at the lactate threshold for all trials.

4.3 Lactate threshold as percentage of ·O_{2max}

Mean ± SD for the percentage ·O_{2max} at the T_{lac} was 69.94 ± 7.39, 75.21 ± 6.84 and 75.93 ± 7.32% for the control, β-alanine and the placebo trial, respectively. The increase in the percentage of ·O_{2max} at the T_{lac} is displayed in figure 4.2. There was a 5.27% increase in the percentage ·O_{2max} in the β-alanine trial and a 5.99% increase in the placebo trial, compared to control trial. A repeated-measures ANOVA revealed that there were no significant differences between the control, β-alanine or the placebo trials (p = 0.087).

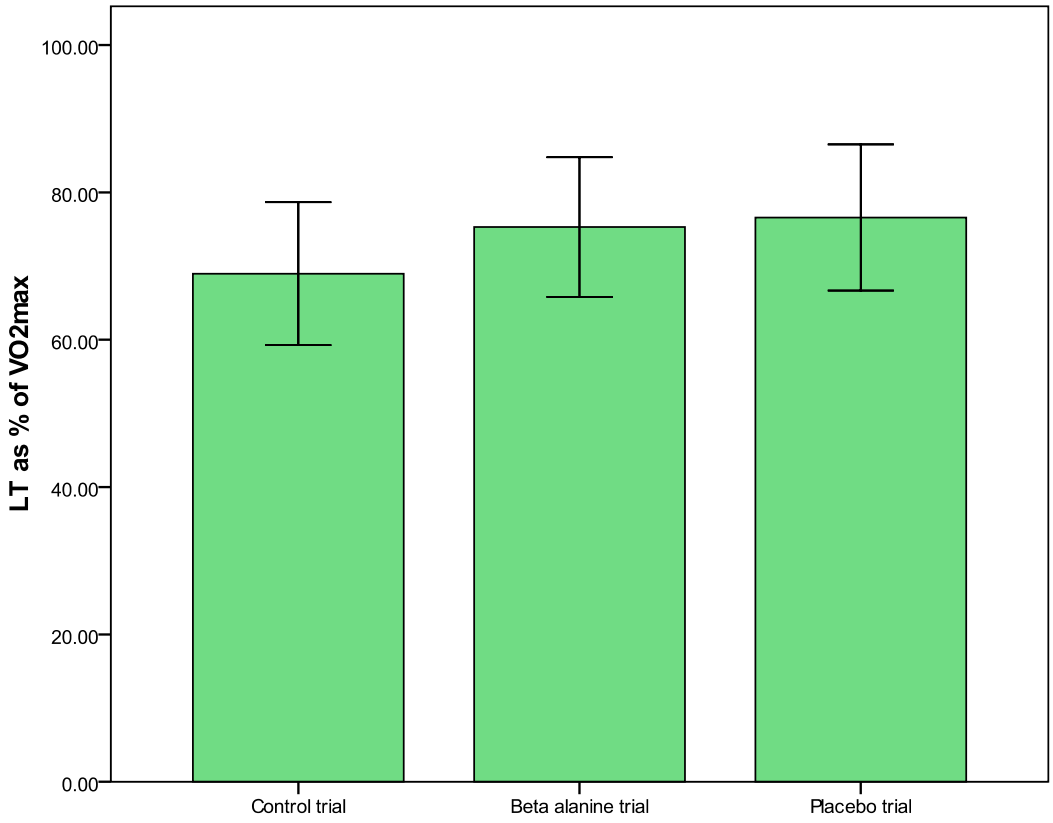

Figure 4.3 Mean ± (SD) of the percentage of $\dot{V}O_{2max}$ at the lactate threshold.

4.4 $\dot{V}O_2$ at the T_{lac}

Mean ± SD for the $\dot{V}O_2$ at the T_{lac} was 37.16 ± 5.68, 40.21 ± 7.63 and 40.38 ± 6.29ml·kg^{-1}·min^{-1} for the control, β-alanine and the placebo trial, respectively. There was a 8.2% increase in the $\dot{V}O_2$ at the T_{lac} in the β-alanine trial and a 8.7% increase in the $\dot{V}O_2$ at the T_{lac} in the placebo trial, compared to the control trial/ The repeated-measures ANOVA revealed that there were no significant variations in $\dot{V}O_2$ values at the T_{lac} between the three trials (p = 0.103). Figure 4.4 shows a boxplot illustrating the spread of data for the $\dot{V}O_2$ at the T_{lac} for all three trials. Participant number 5 is shown as an outliner on the graph due to the unusually low $\dot{V}O_2$ value during the control trial, when compared to the mean.

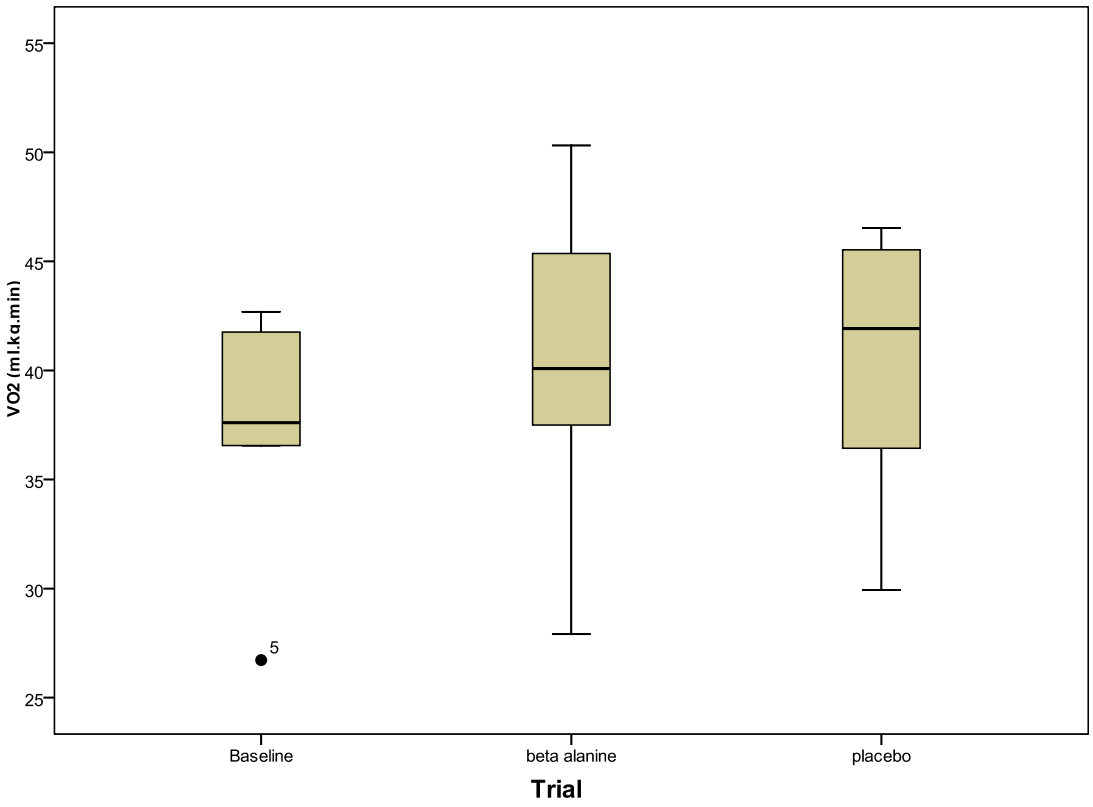

Figure 4.4. Mean ± (SD) of $\dot{V}O_2$ response at the T_{lac} across the three trials.

4.5 Lactate at the T_{lac}

Mean ± SD for the lactate concentrations at the T_{lac} was 4.0 ± 1.8, 4.1 ± 1.2 and 3.7 ± 1.5 mmol·L^{-1} for the control, β-alanine and the placebo trial, respectively. Figure 4.5 shows the individual lactate concentrations at the T_{lac} for the three trials. There was a 10% reduction in lactate levels at the T_{lac} during the placebo trial and a 2.5% increase in lactate concentrations at the T_{lac} in the β-alanine trial, when compared to the control trial. The repeated measures ANOVA uncovered no significant variations in lactate response at the T_{lac} between the three trials (p = 0.628).

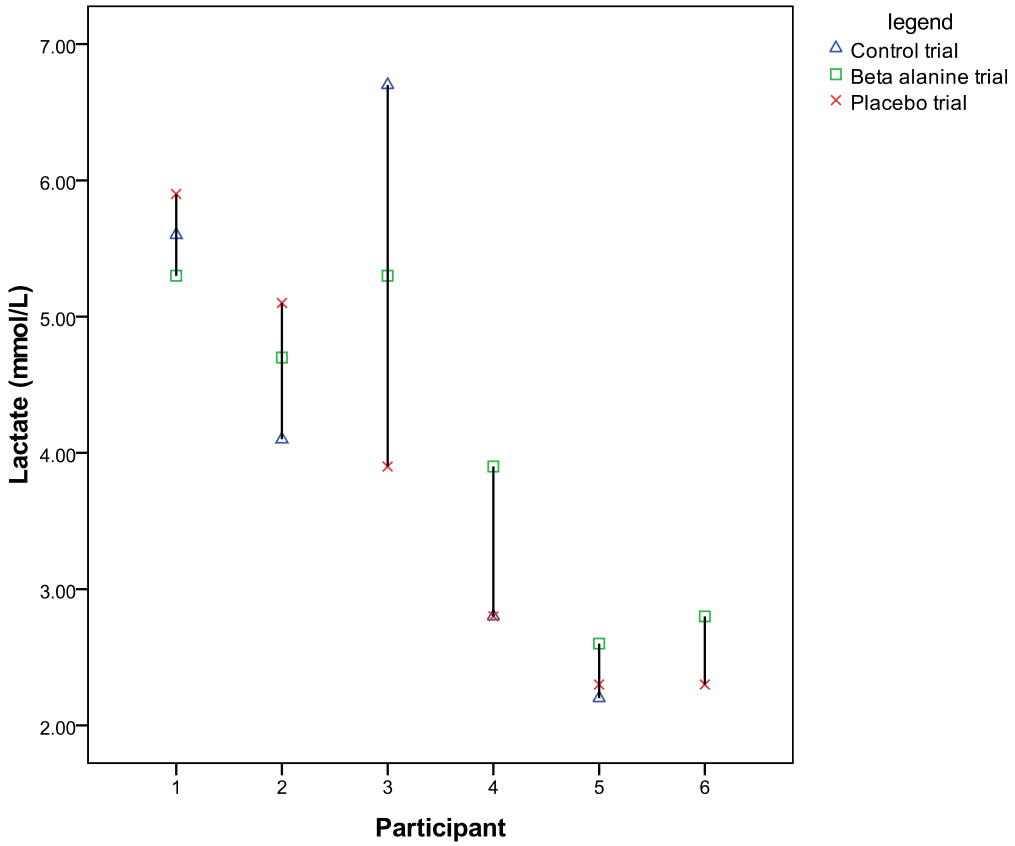

Figure 4.5 Individual participants mean lactate concentrations across the three trials.

4.6 HR at the T_{lac}

Mean ± SD for the HR at the T_{lac} was 168.8 ± 13.9, 165.6 ± 16.3 and 166.4 ± 11.6 beats·min^{-1} for the control, β-alanine and the placebo trials, respectively. There was a 1.9% reduction in the HR at the T_{lac} in the β-alanine trial and a 1.4% reduction in HR at the T_{lac} in the placebo trial, when compared to the control trial. The repeated measures ANOVA revealed no significant variation in HR at the T_{lac} between the three trials (p = 0.626). In 5 out of 6 subjects HR was lower during the β-alanine trial than the placebo or control trials (figure 4.6).

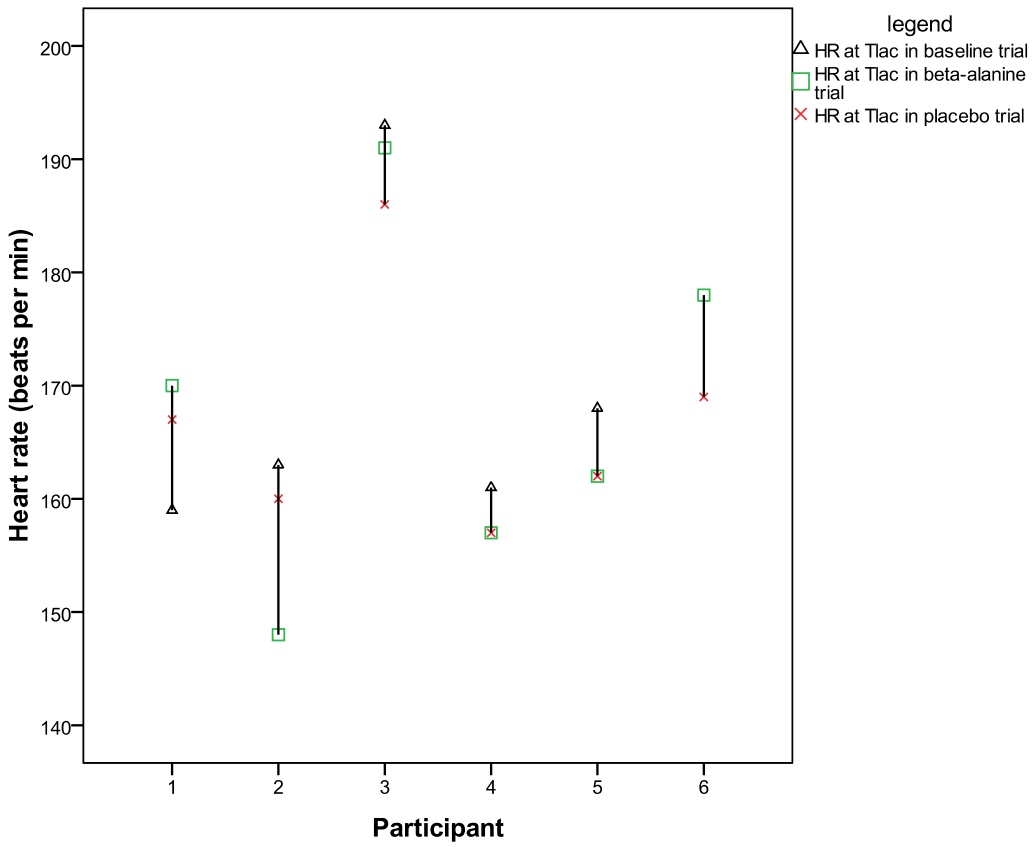

Figure 4.6 Individual heart rate responses at the T_{lac} during the three trials.

4.7 RPE at the T_{lac}

Mean ± SD for the RPE score (6 to 20 scale) at the T_{lac} was 14.4 ± 1.7, 14.0 ± 2.5 and 13.6 ± 2.3 for the control, β-alanine and the placebo trials, respectively. In 3 out of the six participants β-alanine reduced the RPE score at the T_{lac} (figure 4.7). When compared to the control trial, there was a 2.8% reduction in RPE response at the T_{lac} in the β-alanine trial and a 5.6% reduction in RPE response at the T_{lac} in the placebo trial. The repeated measure ANOVA revealed the variation in RPE scores between the three trials was not significant (p = 0.289).

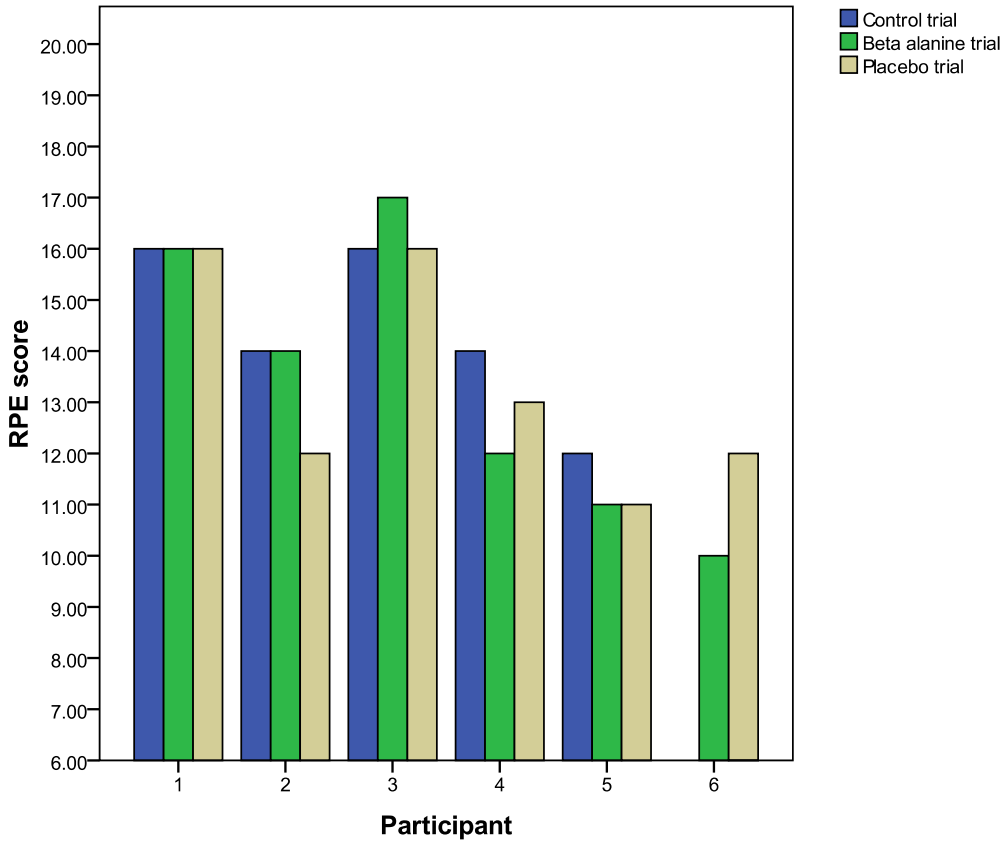

Figure 4.7 Individual RPE response across all trials

5.0. Discussion

The current study is the first to examine the effects of a β-alanine supplementation on various physiological parameters at the T_{lac}, using a five day supplementation protocol. The primary finding is that short term β-alanine supplementation does not have a significant effect on the velocity or the percentage·$\dot{V}O_{2max}$ at the T_{lac}. The primary hypothesis can, therefore, be rejected as five days β-alanine supplementation had no significant effect on the velocity at the T_{lac}. Secondary findings suggest that there is no effect of β-alanine supplementation on percentage of ·$\dot{V}O_{2max}$, ·$\dot{V}O_{2,}$ blood lactate concentrations, HR or RPE at the T_{lac}. This is inconsistent with previous research which reported a significant increase in the power at the T_{lac} (Zoeller et al., 2007) and percentage ·$\dot{V}O_{2max}$ and HR at OBLA (Jordon et al., 2010), following β-alanine supplementation. The discrepancies between the findings of the current study and that of Zoeller et al. (2007) could be related to several factors. Firstly, β-alanine was co-ingested with creatine in the study by Zoeller et al. (2007); whereas in the present study it was ingested alone. Interestingly, in the group that ingested β-alanine on its own there was no effect on ·$\dot{V}O_2$ at the T_{lac}. Secondly, the dose of β-alanine differs between the two studies. The present study experimented with a dose of approximately 3.4g·d^{-1} for five days; in comparison to the 3.2 g·d^{-1} for 28 days used by Zoeller et al (2007). Intramuscular carnosine was not measured in this study or in the study by Zoeller et al (2007). Therefore it cannot be said with any confidence whether muscle carnosine concentrations were amplified or not. Based on previous research that measured muscle carnosine before and after supplementation (Baguet et al., 2009; Derave et al., 2007; Harris et al., 2006; Harris et al., 2009; Hill et al., 2007), carnosine content increased by 37 to 80 percent when supplementing for a period of 4 to 10 weeks. Therefore, it is highly likely that muscle carnosine levels were increased

in the study by Zoeller et al. (2007). The same cannot be assumed in the present study as this was the first to examine the use of a five day dosing protocol. Nevertheless, in a recent study using a 28 day dosing protocol (Baguet et al., 2010) there was no reported influence of β-alanine supplementation on $\dot{V}O_2$ throughout a GXT. Finally, the mode of exercise differs between this study and Zoeller et al. (2007). Beta-alanine supplementation has been reported to be ergogenic in cycling (Hill et al., 2007; Stout et al., 2006; Stout et al., 2007; Stout et al 2008; Smith et al., 2009; Van Thienen et al., 2009; Zoeller et al., 2007), running (Jordon et al., 2010) and rowing (Baguet et al., 2010). With the majority of research being conducted using a cycle ergometer it remains to be elucidated as to whether β-alanine supplementation can delay fatigue in alternative exercise modes.

In research using a running protocol (Jordon et al., 2010), HR and percentage $\dot{V}O_{2max}$ at which OBLA occurred where significantly different following β-alanine supplementation. However, the increase in the percentage $\dot{V}O_{2max}$ at which OBLA occurred is a reflection of the concomitant decrease in $\dot{V}O_{2max}$ and increase in body mass. The results of the study should, therefore, be interpreted with caution. In the present investigation five days β-alanine supplementation had no significant effect on the percentage $\dot{V}O_{2max}$ or HR at the T_{lac}. Four out of the five subjects showed a reduction in HR at the T_{lac} following β-alanine supplementation (figure 4.6). However with closer examination, three out of these four subjects also showed reductions in the velocity at the T_{lac} and, therefore, the reduction in HR is a consequence of a reduced exercise intensity rather than a treatment effect. In the study by Jordon et al. (2010), HR was significantly greater at OBLA in the β-alanine group, whereas there was no difference in the placebo group. The experimental design employed in the present study (repeated measures) favours a stronger statistical comparison compared to a

matched-pairs design due to between-individual variation (Atkinson & Nevill, 1999). Furthermore, the protocol used by Jordon et al. (2010) to assess OBLA is inappropriate for assessing the changes in running performance. The majority of running events are not predominantly ran on a continuous inclination, so the choice to assess OBLA using an incline GXT raises questions as to how effective would β-alanine supplementation would be during level running (Midgley et al., 2007). The author cannot recall any sporting event whereby the gradient is increased every three minutes until the athlete becomes exhausted. In the present investigation, which employed a 1 percent treadmill gradient to represent the energy cost of outdoor running (Jones & Doust, 1996), β-alanine supplementation did not have an effect on HR or percentage $\dot{V}O_{2max}$ at which the T_{lac} occurred.

The main theory explaining the ergogenic effect of β-alanine supplementation proposes that the surplus of β-alanine serves as a precursor for enhanced carnosine synthesis in skeletal muscle thereby augmenting the intramuscular buffering capacity (Harris et al., 2006). In the present investigation the mean lactate concentrations between the three experimental trials were not significantly different ($p < 0.05$). This is in agreement with previous research (Baguet et al., 2010). In the latter investigation, muscle acidosis was attenuated without a concomitant alteration in lactate concentrations. The author concluded that the reduced acidosis represented a better buffering capacity and not a reduction in anaerobic energy delivery. In the current study, despite the non significant differences in lactate concentrations, there was a 2.5 percent increase in lactate levels at the T_{lac} in the β-alanine trial, in conjunction with a reduced velocity at the T_{lac}. This suggests that the ingested β-alanine did not augment the intramuscular buffering capacity in this population of subjects, possibly a consequence of the shorter dosing period employed in the study.

In two out of the six subjects there was a reduction in lactate concentrations at the T_{lac}, but in only one of these subjects was the velocity at the T_{lac} kept constant across the baseline and β-alanine trials. Therefore, the reduced lactate of one of these subjects is likely due to reductions in exercise intensity. On the other hand, variations in carnosine synthetase activity (Tsubone et al., 2007) and/or β-alanine transport (Bakardjiev & Bauer, 1994) between subjects, due to differing training statuses (Parkhouse et al., 1985; Tallon et al., 2005) may mean carnosine synthesis occurs at different rates. It could be postulated that some subjects may possess an enhanced buffering capacity in a shorter amount of time because of these factors. In these select few a shorter dosing period may be adequate to augment muscle carnosine concentrations. Further research is warranted to be able to advise athletes with confidence.

Beta-alanine supplementation had no significant effect on the RPE response at the T_{lac} in the present study. This is similar to the findings of Jordon et al. (2010) who reported no change in RPE at the termination of a GXT following β-alanine supplementation. In contrast, Hoffman et al. (2007) reported a significant ($p \leq 0.05$) decrease in subjective feelings (7-point rating scale, 1 = fresh and 7 = tired) of fatigue in 26 college American football players following 30 days β-alanine supplementation when compared to a placebo group (3.96 ± 0.80 vs. 4.55 ± 0.83). The validity and reliability of the 7-point rating scale could be questioned and partly explain the significant finding on perceived fatigue. The present study and that of Jordon et al. (2010) used a traditional RPE scale of 6 to 20, which is valid and reliable (Borg, 1998). It should be noted that the aerobic nature of the exercise performed in the current study is in contrast to the anaerobic type of exercise assessed in the study by Hoffman et al. (2007). On the assumption that an all-out effort was required for the

Wingate test, the line drills and during the resistance training programme, any increase in carnosine concentrations would serve to reduce feeling of fatigue in this type of exercise, for the same absolute exercise intensity. However, the reduced perception of practice intensity in the β-alanine group (3.92 ± 0.60, 1 to 7 scale) compared to the placebo group (4.18 ± 0.40), although not significant ($p > 0.05$), may represent a reduced effort during training and consequently reduced feelings of fatigue.

The inconstancies highlighted between this research and that from other laboratories (Baguet et al., 2010; Hoffman et al., 2007; Jordon et al., 2010; Zoeller et al., 2007) are partly related to variation in exercise type, participant training status, experimental design and supplement dose. It is, therefore, important to highlight some limitations in the present study that may have affected the outcome. Firstly, muscle carnosine concentrations were not measured at any point during the investigation. Several recently published studies (Baguet et al., 2010; Jordon et al., 2010; Sweeney et al., 2010) also neglected to measure carnosine concentration during the course of their studies. However, previous clinical trials (Bageut et al., 2009; Derave et al., 2007; Harris et al., 2006; Harris et al., 2009; Hill et al., 2007) assessing β-alanine supplementation have measured intramuscular carnosine content before and after the supplementation period, using the biopsy technique or using proton magnetic resonance spectroscopy. In all these studies intramuscular carnosine concentrations were enhanced by 27 to 80 percent. The large variation in the response can be attributed to the type of the muscle sampled, the dose and length of supplementation and the training status of the participants. Nevertheless, all subjects reported improvements in carnosine concentrations to some degree. The majority of studies (Bageut et al., 2009; Baguet et al., 2010; Derave et al., 2007; Harris et al., 2006;

Harris et al., 2009; Hill et al., 2007; Hoffman et al., 2007; Jordon et al., 2010; Stout et al., 2006; Stout et al., 2007; Stout et al., 2008; Sweeney et al., 2010; Van Theinen et al., 2009; Zoeller et al., 2007) utilised, at least, a 28 day supplementation period. Some studies have demonstrated an ergogenic effective of β-alanine supplementation employing a 14 day protocol (Harris et al., 2006; Ponte et al., 2007). Although intramuscular carnosine concentrations were not measured in these investigations, significant reductions in fatigue were noted. In the present study, the effects of a five day supplementation period were assessed. Having not measured intramuscular carnosine concentrations it cannot be said with any certainty whether this supplementation period is sufficient to increase carnosine concentrations. Based on the exploratory work of Harris et al. (2006) 28 days of β-alanine supplementation ($3.2g \cdot d^{-1}$) was suffice to elicit a 42 percent increase in muscle carnosine concentrations. This equates to a 1.5 percentage increase in muscle carnosine concentrations per day. The average dose in the present study was $3.4g \cdot d^{-1}$ so in theory participants could potentially have enhanced carnosine concentrations by at least 7.5 percent. However, without measuring intramuscular carnosine concentrations this cannot be confirmed. It could be hypothesised that an increase of such magnitude was insufficient to enhance the buffering capacity of the active musculature, which typically stands between 7 and 15 percent of the total muscle buffering capacity (Harris et al., 2006; Mannion et al., 1995; Tallon et al., 2005).

Another limitation in the present study relates to the three week washout period encompassed within the experimental design. This is a repercussion of not knowing, with any conviction, how much a shorter β-alanine supplementation period augments muscle carnosine content. It is, therefore, difficult to estimate how much carnosine was hydrolysed during the three week washout period. Intramuscular

carnosine is a relatively stable compound due to the lack of the carnosine dipeptiase activity in muscle cells (Otani et al., 2005). Bageut et al. (2009) divided participants into high (n =3) and low responders (n = 5) with regards to carnosine increases and washout response. The high responders demonstrated a 55 percent increase in muscle carnosine content and showed a washout response of 3.5 percent per week. The low responders showed a 15 percent increase in carnosine content and a washout rate of 2.5 percent per week. In terms of time, this corresponded to a washout period of 15 and 7 weeks for the high and low responders, respectively. If the proposed increase in carnosine content (≥7.5 percent) held true in the present study, and all participants were low responders, it would take approximately three weeks to hydrolyse the supplementation-induced carnosine increases. Thereby, muscle carnosine content would be at pre-supplementation levels for the next testing session. Therefore, in theory a three week washout period should be sufficient to eliminate any supplementation-induced changes in muscle carnosine content, although further work is warranted in regards to carnosine dynamics and individual response. There has been no work to date that has assessed the impact of training on muscle carnosine hydrolysis. The participants in the study by Baguet et al. (2010) were reported to be "*physically active but not involved in regular training.*" Two of the participants in the present study were soccer players who began pre-season training during the course of the study. Consequently carnosine synthesis rate (Parkhouse et al., 1985; Tallon et al., 2005) and physiological response during the subsequent T_{lac} test (Smith et al., 2009; Smith et al., 2009) may have been a result of training induced adaptations rather than a response to β-alanine supplementation. Furthermore, training may have affected carnosine washout rate. If training increases carnosine synthesis (Tallon et al., 2005) it could be possible that the washout rate is concomitantly reduced. If this were the

case then this may have had implications during subsequent testing session after the three week washout period.

There are limited side effects reported in the literature following β-alanine ingestion. A harmless, yet commonly occurring, side effect is paresthesia (Artioli et al., 2010; Derave et al., 2010). When single doses in excess of 800mg are consumed, symptoms of paresthesia are reported 20 to 25 minutes after ingestion and subside within approximately one hour. This was first reported by the intuitive work of Harris et al. (2006); surprisingly however, few labortatories have reported treatment blinding issues during investigations. In the present study, three out of the six participants reported symptoms of paresthesia following ingestion of β-alanine, despite not ingesting more than two capsules (< 800mg) in a single dose. Therefore, the double-blinding of treatments was unsuccessful during this investigation and may have impacted on the results. Recently, a controlled release formulation of β-alanine was trialled to attempt to reduce the reported symptoms of paresthesia (Harris et al., 2009). Muscle carnosine was augmented by 40 percent after four weeks of supplementation and participants ingested 1600mg in a single dose without any symptoms of paresthesia. This may improve compliance during investigations and make supplementing more practical as fewer doses will be required. For example, one participant in the current study had to take > 10 capsules each day, interspersed with at least two hours between doses. If this had been a ten week supplementation period, as employed in some investigations (Hill et al., 2007), compliance may not have been as good. Participants in the current study verbally confirmed that their compliance with supplementation was 100 percent.

Participants in the current study were asked to maintain their usual dietary practices and replicate them in the 48 hours prior to each testing session. This was

assessed via verbal confirmation from the participant before each session. It can therefore, be assumed that dietary intake of carnosine and β-alanine was kept relatively stable during the testing period. One of the participants was a pescetarian and given the influence of meat ingestion on carnosine content (Harris et al., 2007) this participant may have had lower carnosine concentrations to begin with and so may therefore be expected to respond to a greater extent following β-alanine supplementation. Some fish (tuna and mackerel) are reported to have greater dipeptide content than poultry and meat (Abe, 2000) suggesting that carnosine concentrations in pescetarians may not be all that different from omnivores (Harris et al., 2007). Furthermore, the lack of a significant positive correlation between carnosine content and meat ingestion reported by Baguet et al. (2009) emphasises that diet does not greatly affect muscle carnosine content.

Another apparent limitation in the current investigation is the sample size. A previous power calculation estimated that for an effect size of 0.20 (i.e. detection of the smallest worthwhile effects, 20%) and an alpha level of 0.05 (detection of non existent effects, 5%), 27 participants would be required to enable significant differences to be identified. Based on the variation about the means and the SD across the trials for velocity at the $T_{lac,}$ it is calculated that 57 participants would be required to detect the smallest worthwhile change in the velocity at the $T_{lac.}$ In addition, this investigation utilised a 1 $km \cdot h^{-1}$ increase in treadmill velocity every three minuets. This protocol may not be sensitive enough to detect changes in the velocity at T_{lac} (Faude et al., 2009). Therefore, with a greater sample size and a protocol with smaller increments in treadmill velocity at each stage, short term β-alanine supplementation may prove to be ergogenic at the T_{lac} during treadmill running.

5.1. Recommendation for future research

Research on β-alanine supplementation is still in it infancy. The results of the present study indicate that a shorter dosing period is ineffective at improving the T_{lac} in recreationally active individuals. Future research should attempt to elucidate the optimum dosing strategy to maximise muscle carnosine concentrations and as to what extent this augments muscle buffering capacity and exercise performance. At present the diversity across studies in the supplement amount and the length of the dosing period makes prescribing the optimum dosing protocol difficult. Shorter supplementation periods (< 14 days) should be explored to examine the effect on muscle carnosine conconcentrations. This would make supplementing more practical for athletes and exercise enthusiasts. Research with athletes holds promise (Derave et al., 2007; Van Thienen et al., 2009). More work is warranted on β-alanine supplementation and carnosine dynamics in high-level athletes to depict if carnosine levels are increased to the same degree as in less conditioned individuals. The effect of long term training could influence the rate of carnosine synthesis subsequent to exercise performance (Smith et al., 2009). In addition, the effect of training on carnosine washout following β-alanine supplementation is an area still unexplored. Further work to assess the dynamics of carnosine after β-alanine supplementation-induced increases would enable more quality repeated-measures, placebo controlled, cross-over design research studies to be conducted; thereby allowing stronger statistical comparisons to be made. Future research needs to examine the effects of β-alanine supplementation and muscle carnosine content across different sports and assess its effect on performance. Extending our knowledge about the physiological diversity of carnosine (Begum et al., 2005) would help to determine which exercise situations β-alanine supplementation would provide the greatest ergogenic effect.

With only one side effect being reported with β-alanine supplementation it appears that the short term side effects are sparse and are not of any harm to health. On the other hand the long term (>10 weeks) health consequences of β-alanine supplementation are presently unknown. Given that β-alanine is a naturally occurring amino acid found abundantly in the tissue of animals, it is likely to be safe (Artioli et al., 2010). Nevertheless, future studies using a ten week plus supplementation period need to share any adverse effects reported by participants.

5.2 Conclusion

It can be concluded from the current data that five days β-alanine supplementation has no ergogenic effect on the velocity or the percentage of $\dot{V}O_{2max}$ at the T_{lac} during treadmill running. Participants undertook three T_{lac} test on separate occasions. One was a control trial and the other two were following a five day supplementation period with 50mg.kg-1 of β-alanine or a placebo. There was no significant difference in the velocity at the T_{lac} between the control, the placebo or the β-alanine trial. Likewise, there was no significant difference in percentage of $\dot{V}O_{2max}$ following β-alanine supplementation when compared to the placebo or control trial. Therefore, the primary hypothesis can be rejected. This suggests that either five day β-alanine supplementation does not allow sufficient time to synthesise and augment intramuscular carnosine concentrations, or that the exercise-induced increases in acidosis at the T_{lac} can be buffered with normal physiological concentrations of carnosine, and further carnosine increases fail to enhance the T_{lac}. Furthermore, there were no significant differences in the $\dot{V}O_2$, lactate concentrations, heart rate or RPE at

the T_{lac}. Collectively, these results suggest that β-alanine is not an effective performance enhancing nutritional supplement for long distance running. Further research in which a typical 28 day supplementation period is employed is warranted to support or refute the results of the present investigation.

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7. Appendices

Appendix A

CONSENT FORM

Effect of Beta Alanine Supplementation on the Lactate Threshold during Treadmill Running

Please tick the box if you agree with the statement:

1. I confirm that I have read and understood the participant information sheet for the above-named study, and have had the opportunity to ask the lead researcher any questions. ☐

2. I understand that my participation is voluntary, and that I am free to withdraw from participating in the study at any time, without giving any reason and without my rights being affected. ☐

3. I agree that the data collected from the study can be used in the writing of a scientific report and should the report be deemed creditable the data used in the report can be published. ☐

4. I agree to take part in the above study. ☐

Name of participant ……

Date ……

Signed ……

Name of researcher ……

Date ……

Signed …….

Appendix B

Pre-test Health Questionnaire

Personal Information

Name:_____ Test date:

Address:_____

Contact number:_____ Date of birth:_____

In order to ensure that the physiological assessments are as safe and accurate as possible, it is important that each participant is screened for any factors that may influence the results. Please circle your answer to the following questions:

1. Has your doctor ever said that you have a heart condition *and* that you should only perform physical activity recommended by a doctor? YES/NO

2. Do you feel pain in the chest when you perform physical activity? YES/NO

3. In the past month, have you had chest pain when you were not performing physical activity? YES/NO

4. Do you lose your balance because of dizziness *or* do you ever lose consciousness? YES/NO

5. Do you have bone or joint problems (e.g. back, knee or hip) that could be made worse by a change in your physical activity? YES/NO

6. Is your doctor currently prescribing any medication for you? YES/NO

   If yes please provide details:_____
   _____
   _____
   _____

7. Are you pregnant, or have you been pregnant in the last six months? YES/NO

8. Have you injured your hip, knee or ankle joint in the last six months? YES/NO

9. Do you know of any other reason why you should not participate in physical activity? YES/NO

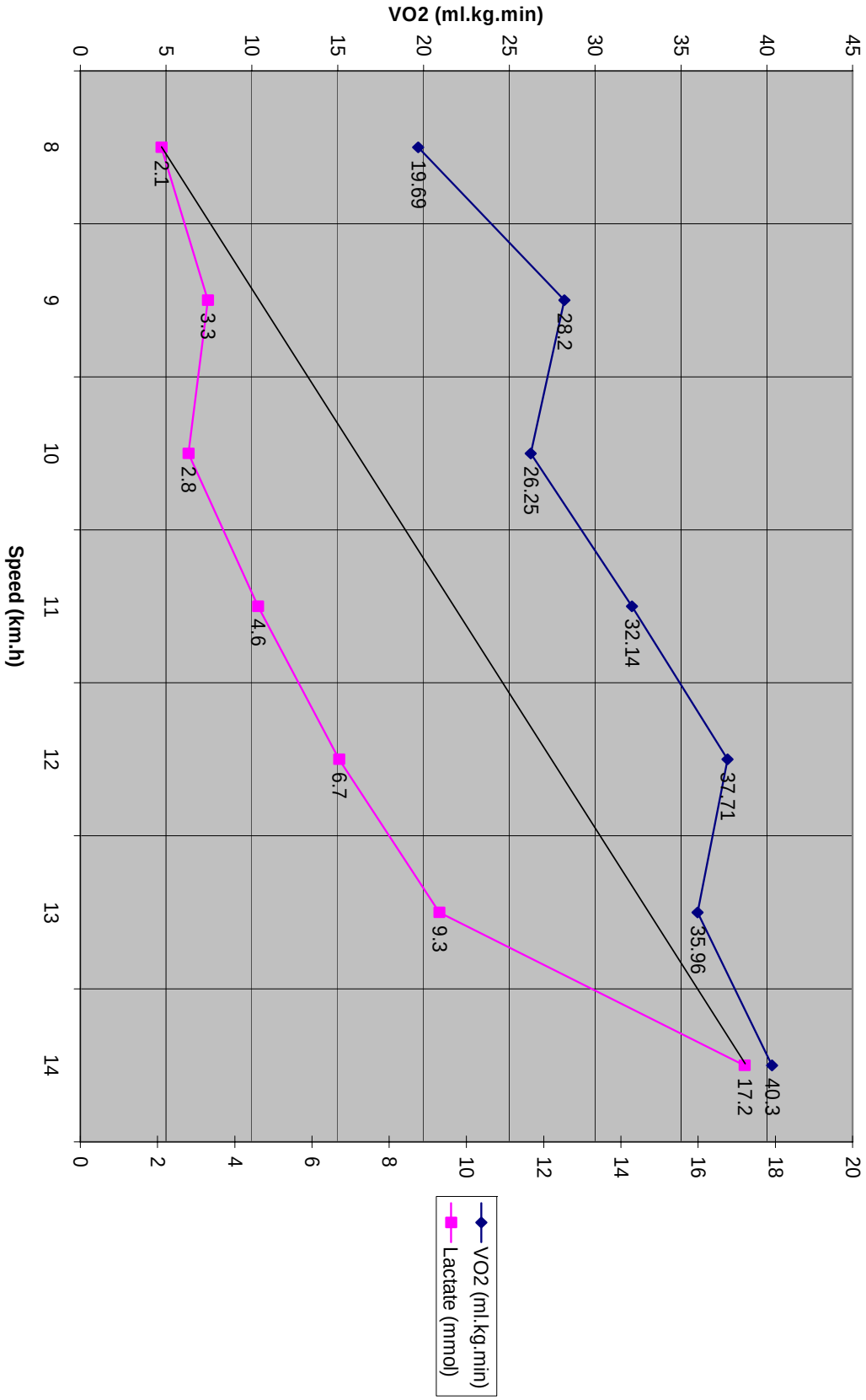
Appendix C
RPE instructions

Borg (1998) provides specific instructions for the use of his 6 – 20 RPE scale which should be reiterated to participants during each exercise session. He states:

> "While exercising we want you to rate your perception of exertion, i.e., how heavy and strenuous the exercise feels to you. The perception of exertion depends mainly on the strain and fatigue in your muscles and on your feeling of breathlessness or aches in the chest.
>
> Look at this rating scale; we want you to use this scale from 6 to 20, where 6 means "no exertion at all" and 20 means "maximal exertion."
>
> 9 corresponds to "very light" exercise. For a normal healthy person it is like walking slowly at his or her own pace for some minute.
>
> 13 on the scale is "somewhat hard" exercise, but it still feels OK to continue.
>
> 17 "very hard" is very strenuous. A healthy person can still go on, but he or she really has to push him- or herself. It feels vary heavy, and the person is tired.
>
> 19 on the scale is an extremely strenuous exercise level. For most people this is the most strenuous exercise they have ever experienced.
>
> Try to appraise your feelings of exertion as honestly as possible, without thinking about what the actual physical load is. Don't underestimate it, but don't overestimate it either. It's your own feeling of effort and exertion that's important, not how it compares to other people's. What other people think is not important either. Look at the scale and the expressions and then give a number.
>
> Any questions?"

This will be used to inform participants of how to use the RPE scale for every exercise testing session.

Appendix D
Lactate threshold graph



Appendix E

Certificate of Analysis

Beta Alanine

[redacted]

CERTIFICATE OF ANALYSIS

PRODUCT NAME **BETA-ALANINE** [redacted]

[redacted]

Manufacture Date	March 2010
Re-test Date	March 2013

TEST	SPECIFICATION	RESULTS
Appearance	Crystal Powder	Conforms
Colour	White/slightly yellow	White or Slightly Yellow
Assay	98.0 – 101.0%	98.56%
Ammonium	Max 0.02%	Conforms
Loss on Drying	Max 0.30%	0.23%
Chloride	Max 0.04%	Conforms
Residue on Ignition	Max 0.2%	0.08%
Sulphate	Max 0.048%	< 0.048%
pH	6.5 – 7.5	6.75
GMO Status	Non	Non GMO
Irradiation Status	Non	Non Irradiated

We confirm that the above is a true copy of the original manufacturer's/supplier's Certificate of Analysis.

[redacted]

Appendix F

University of Chester

$\dot{V}O_{2max}/T_{lac}$ Treadmill Protocol Data Collection Sheet

Participant: _____ D.O.B: _____

Date: _____

Session: Signed consent form: YES/NO

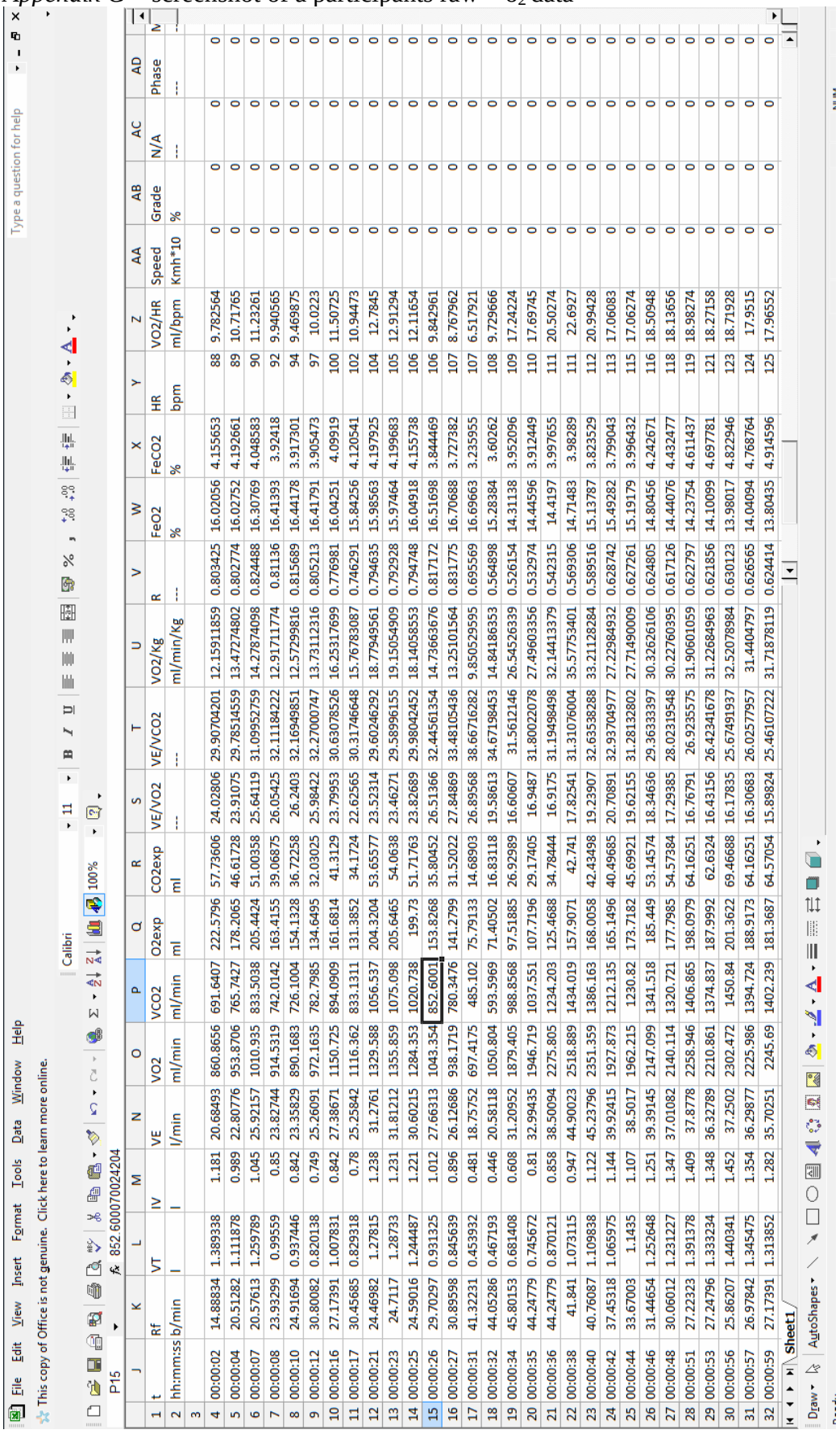
Height (cm): Weight (Kg):

BMI: BP:

NB each stage lasts 3 minutes

Stage	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Speed (Km/h)							
Gradient (%)	1.0	1.0	1.0	1.0	1.0	1.0	1.0
HR (bpm)							
$\dot{V}O_2$ ($ml \cdot kg^{-1} \cdot min^{-1}$)							
RPE							
Lactate (mM)							
RER							

Appendix G – screenshot of a participants raw o_2 data



	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z	AA	AB	AC	AD
1	t	Rf	VT	IV	VE	VO2	VCO2	O2exp	CO2exp	VE/VO2	VE/VCO2	VO2/kg	R	FeO2	FeCO2	HR	VO2/HR	Speed	Grade	N/A	Phase
2	hh:mm:ss	b/min	l	l	l/min	ml/min	ml/min	ml	ml	---	---	ml/min/kg	---	%	%	bpm	ml/bpm	Kmh*10	%	---	---
3																					
4	00:00:02	14.88834	1.389338	1.181	20.68493	860.8656	691.6407	222.5796	57.73606	24.02806	29.90704201	12.15911859	0.803425	16.02056	4.155653	88	9.782564	0	0	0	0
5	00:00:04	20.51282	1.111878	0.989	22.80776	953.8706	765.7427	178.2065	46.61728	23.91075	29.78514559	13.47274802	0.802774	16.02752	4.192661	89	10.71765	0	0	0	0
6	00:00:07	20.57613	1.259789	1.045	25.92157	1010.935	833.5038	205.4424	51.00358	25.64119	31.09952759	14.27874098	0.824488	16.30769	4.048583	90	11.23261	0	0	0	0
7	00:00:08	23.93299	0.99559	0.85	23.82744	914.5319	742.0142	163.4155	39.06875	26.05425	32.11184222	12.91711774	0.81136	16.41393	3.92418	92	9.940565	0	0	0	0
8	00:00:10	24.91694	0.937446	0.842	23.35829	890.1683	726.1004	154.1328	36.72258	26.2403	32.16949851	12.57299816	0.815689	16.44178	3.917301	94	9.469875	0	0	0	0
9	00:00:12	30.80082	0.820138	0.749	25.26091	972.1635	782.7985	134.6495	32.03025	25.98422	32.27000747	13.73112316	0.805213	16.41791	3.905473	97	10.0223	0	0	0	0
10	00:00:16	27.17391	1.007831	0.842	27.38671	1150.725	894.0909	161.6814	41.3129	23.79953	30.63078526	16.25317699	0.776981	16.04251	4.09919	100	11.50725	0	0	0	0
11	00:00:17	30.45685	0.829318	0.78	25.25842	1116.362	833.1311	131.3852	34.1724	22.62565	30.31746648	15.76783087	0.746291	15.84256	4.120541	102	10.94473	0	0	0	0
12	00:00:21	24.46982	1.27815	1.238	31.2761	1329.588	1056.537	204.3204	53.65577	23.52314	29.60246292	18.77949561	0.794635	15.98563	4.197925	104	12.7845	0	0	0	0
13	00:00:23	24.7117	1.28733	1.231	31.81212	1355.859	1075.098	205.6465	54.0638	23.46271	29.58996155	19.15054909	0.792928	15.97464	4.199683	105	12.91294	0	0	0	0
14	00:00:25	24.59016	1.244487	1.221	30.60215	1284.353	1020.738	199.73	51.71763	23.82689	29.98042452	18.14058553	0.794748	16.04918	4.155738	106	12.11654	0	0	0	0
15	00:00:26	29.70297	0.931325	1.012	27.66313	1043.354	852.6001	153.8268	35.80452	26.51366	32.44561354	14.73663676	0.817172	16.51698	3.844469	106	9.842961	0	0	0	0
16	00:00:27	30.89598	0.845639	0.896	26.12686	938.1719	780.3476	141.2799	31.52022	27.84869	33.48105436	13.25101564	0.831775	16.70688	3.727382	107	8.767962	0	0	0	0
17	00:00:31	41.32231	0.453932	0.481	18.75752	697.4175	485.102	75.79133	14.68903	26.89568	38.66716282	9.850529595	0.695569	16.69663	3.235955	107	6.517921	0	0	0	0
18	00:00:32	44.05286	0.467193	0.446	20.58118	1050.804	593.5969	71.40502	16.83118	19.58613	34.67198453	14.84186353	0.564898	15.28384	3.60262	108	9.729666	0	0	0	0
19	00:00:34	45.80153	0.681408	0.608	31.20952	1879.405	988.8568	97.51885	26.92989	16.60607	31.5612146	26.54526339	0.526154	14.31138	3.952096	109	17.24224	0	0	0	0
20	00:00:35	44.24779	0.745672	0.81	32.99435	1946.719	1037.551	107.7196	29.17405	16.9487	31.80022078	27.49603356	0.532974	14.44596	3.912449	110	17.69745	0	0	0	0
21	00:00:36	44.24779	0.870121	0.858	38.50094	2275.805	1234.203	125.4688	34.78444	16.9175	31.19498498	32.14413379	0.542315	14.4197	3.997655	111	20.50274	0	0	0	0
22	00:00:38	41.841	1.073115	0.947	44.90023	2518.889	1434.019	157.9071	42.741	17.82541	31.31076004	35.57753401	0.569306	14.71483	3.98289	111	22.6927	0	0	0	0
23	00:00:40	40.76087	1.109838	1.122	45.23796	2351.359	1386.163	168.0058	42.43498	19.23907	32.63538288	33.21128284	0.589516	15.13787	3.823529	112	20.99428	0	0	0	0
24	00:00:42	37.45318	1.065975	1.144	39.92415	1927.873	1212.135	165.1496	40.49685	20.70891	32.93704977	27.22984932	0.628742	15.49282	3.799043	113	17.06083	0	0	0	0
25	00:00:44	33.67003	1.1435	1.107	38.5017	1962.215	1230.82	173.7182	45.69921	19.62155	31.28132802	27.71490009	0.627261	15.19179	3.996432	115	17.06274	0	0	0	0
26	00:00:46	31.44654	1.252648	1.251	39.39145	2147.099	1341.518	185.449	53.14574	18.34636	29.36333397	30.32626106	0.624805	14.80456	4.242671	116	18.50948	0	0	0	0
27	00:00:48	30.06012	1.231227	1.347	37.01082	2140.114	1320.721	177.7985	54.57384	17.29385	28.02319548	30.22760395	0.617126	14.44076	4.432477	118	18.13656	0	0	0	0
28	00:00:51	27.22323	1.391378	1.409	37.8778	2258.946	1406.865	198.0979	64.16251	16.76791	26.9235575	31.90601059	0.622797	14.23754	4.611437	119	18.98274	0	0	0	0
29	00:00:53	27.24796	1.333234	1.348	36.32789	2210.861	1374.837	187.9992	62.6324	16.43156	26.42341678	31.22684963	0.621856	14.10099	4.697781	121	18.27158	0	0	0	0
30	00:00:56	25.86207	1.440341	1.452	37.2502	2302.472	1450.84	201.3622	69.46688	16.17835	25.67491937	32.52078984	0.630123	13.98017	4.822946	123	18.71928	0	0	0	0
31	00:00:57	26.97842	1.345475	1.354	36.29877	2225.986	1394.724	188.9173	64.16251	16.30683	26.02577957	31.4404797	0.626565	14.04094	4.768764	124	17.9515	0	0	0	0
32	00:00:59	27.17391	1.313852	1.282	35.70251	2245.69	1402.239	181.3687	64.57054	15.89824	25.46107222	31.71878119	0.624414	13.80435	4.914596	125	17.96552	0	0	0	0

Appendix H – SPSS data

Velocity at the lactate threshold

Mauchly's Test of Sphericity[b]

Measure:MEASURE_1

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon[a]		
					Greenhouse-Geisser	Huynh-Feldt	Lower-bound
VelcocityatLT	.745	1.180	2	.554	.797	1.000	.500

Tests the null hypothesis that the error covariance matrix of the orthonormalized transformed dependent variables is proportional to an identity matrix.

a. May be used to adjust the degrees of freedom for the averaged tests of significance. Corrected tests are displayed in the Tests of Within-Subjects Effects table.

b. Design: Intercept
Within Subjects Design: VelcocityatLT

Tests of Within-Subjects Effects

Measure:MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
VelcocityatLT	Sphericity Assumed	1.444	2	.722	1.102	.369
	Greenhouse-Geisser	1.444	1.593	.907	1.102	.362
	Huynh-Feldt	1.444	2.000	.722	1.102	.369
	Lower-bound	1.444	1.000	1.444	1.102	.342
Error(VelcocityatLT)	Sphericity Assumed	6.556	10	.656		
	Greenhouse-Geisser	6.556	7.966	.823		
	Huynh-Feldt	6.556	10.000	.656		
	Lower-bound	6.556	5.000	1.311		

Percentage of o_{2max} at the lactate threshold

Mauchly's Test of Sphericity[b]

Measure:MEASURE_1

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon[a]		
					Greenhouse-Geisser	Huynh-Feldt	Lower-bound
percentVO2max	.755	1.126	2	.570	.803	1.000	.500

Tests the null hypothesis that the error covariance matrix of the orthonormalized transformed dependent variables is proportional to an identity matrix.

a. May be used to adjust the degrees of freedom for the averaged tests of significance. Corrected tests are displayed in the Tests of Within-Subjects Effects table.

b. Design: Intercept
Within Subjects Design: percentVO2max

Tests of Within-Subjects Effects

Measure:MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
percentVO2max	Sphericity Assumed	128.613	2	64.306	3.141	.087
	Greenhouse-Geisser	128.613	1.606	80.079	3.141	.104
	Huynh-Feldt	128.613	2.000	64.306	3.141	.087
	Lower-bound	128.613	1.000	128.613	3.141	.137
Error(percentVO2max)	Sphericity Assumed	204.746	10	20.475		
	Greenhouse-Geisser	204.746	8.030	25.497		
	Huynh-Feldt	204.746	10.000	20.475		
	Lower-bound	204.746	5.000	40.949		

o_2 at the lactate threshold

Mauchly's Test of Sphericity[b]

Measure:MEASURE_1

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon[a]		
					Greenhouse-Geisser	Huynh-Feldt	Lower-bound
VO2	.720	1.312	2	.519	.781	1.000	.500

Tests the null hypothesis that the error covariance matrix of the orthonormalized transformed dependent variables is proportional to an identity matrix.

a. May be used to adjust the degrees of freedom for the averaged tests of significance. Corrected tests are displayed in the Tests of Within-Subjects Effects table.

b. Design: Intercept
Within Subjects Design: VO2

Tests of Within-Subjects Effects

Measure:MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
VO2	Sphericity Assumed	39.419	2	19.709	2.875	.103
	Greenhouse-Geisser	39.419	1.563	25.222	2.875	.122
	Huynh-Feldt	39.419	2.000	19.709	2.875	.103
	Lower-bound	39.419	1.000	39.419	2.875	.151
Error(VO2)	Sphericity Assumed	68.547	10	6.855		
	Greenhouse-Geisser	68.547	7.815	8.772		
	Huynh-Feldt	68.547	10.000	6.855		
	Lower-bound	68.547	5.000	13.709		

Lactate at the lactate threshold

Mauchly's Test of Sphericity[b]

Measure:MEASURE_1

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon[a]		
					Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Lactate	.555	2.352	2	.308	.692	.872	.500

Tests the null hypothesis that the error covariance matrix of the orthonormalized transformed dependent variables is proportional to an identity matrix.

a. May be used to adjust the degrees of freedom for the averaged tests of significance. Corrected tests are displayed in the Tests of Within-Subjects Effects table.

b. Design: Intercept
Within Subjects Design: Lactate

Tests of Within-Subjects Effects

Measure:MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Lactate	Sphericity Assumed	.503	2	.252	.487	.628
	Greenhouse-Geisser	.503	1.384	.364	.487	.568
	Huynh-Feldt	.503	1.744	.289	.487	.605
	Lower-bound	.503	1.000	.503	.487	.516
Error(Lactate)	Sphericity Assumed	5.163	10	.516		
	Greenhouse-Geisser	5.163	6.922	.746		
	Huynh-Feldt	5.163	8.722	.592		
	Lower-bound	5.163	5.000	1.033		

Heart rate at the lactate threshold

Mauchly's Test of Sphericity[b]

Measure:MEASURE_1

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon[a]		
					Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Heartrate	.671	1.199	2	.549	.752	1.000	.500

Tests the null hypothesis that the error covariance matrix of the orthonormalized transformed dependent variables is proportional to an identity matrix.

a. May be used to adjust the degrees of freedom for the averaged tests of significance. Corrected tests are displayed in the Tests of Within-Subjects Effects table.

b. Design: Intercept
Within Subjects Design: Heartrate

Tests of Within-Subjects Effects

Measure:MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Heartrate	Sphericity Assumed	27.733	2	13.867	.496	.626
	Greenhouse-Geisser	27.733	1.504	18.435	.496	.582
	Huynh-Feldt	27.733	2.000	13.867	.496	.626
	Lower-bound	27.733	1.000	27.733	.496	.520
Error(Heartrate)	Sphericity Assumed	223.600	8	27.950		
	Greenhouse-Geisser	223.600	6.017	37.158		
	Huynh-Feldt	223.600	8.000	27.950		
	Lower-bound	223.600	4.000	55.900		

RPE at the lactate threshold

Mauchly's Test of Sphericity[b]

Measure:MEASURE_1

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon[a]		
					Greenhouse-Geisser	Huynh-Feldt	Lower-bound
RPE	.868	.425	2	.808	.883	1.000	.500

Tests the null hypothesis that the error covariance matrix of the orthonormalized transformed dependent variables is proportional to an identity matrix.

a. May be used to adjust the degrees of freedom for the averaged tests of significance. Corrected tests are displayed in the Tests of Within-Subjects Effects table.

b. Design: Intercept
Within Subjects Design: RPE

Tests of Within-Subjects Effects

Measure:MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
RPE	Sphericity Assumed	1.600	2	.800	1.455	.289
	Greenhouse-Geisser	1.600	1.766	.906	1.455	.292
	Huynh-Feldt	1.600	2.000	.800	1.455	.289
	Lower-bound	1.600	1.000	1.600	1.455	.294
Error(RPE)	Sphericity Assumed	4.400	8	.550		
	Greenhouse-Geisser	4.400	7.066	.623		
	Huynh-Feldt	4.400	8.000	.550		
	Lower-bound	4.400	4.000	1.100		